Statement of Co-Authorship

The following people and institutions contributed to the publication of the work undertaken as part of this thesis:

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Pollination Ecology and Evolution of Epacrids

by

Karen A. Johnson BSc (Hons)

Submitted in fulfilment of the requirements for the Degree of Doctor of Philosophy

University of Tasmania

February 2012

Declaration of originality

This thesis contains no material which has been accepted for the award of any other degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Abstract

Relationships between plants and their pollinators are thought to have played a major role in the morphological diversification of angiosperms. The epacrids (subfamily Styphelioideae) comprise more than 550 species of woody plants ranging from small prostrate shrubs to temperate rainforest emergents. Their range extends from SE Asia through Oceania to Tierra del Fuego with their highest diversity in Australia. The overall aim of the thesis is to determine the relationships between epacrid floral features and potential pollinators, and assess the evolutionary status of any pollination syndromes. The main hypotheses were that flower characteristics relate to pollinators in predictable ways; and that there is convergent evolution in the development of pollination syndromes.

Four case-studies obtained information on specific epacrid-pollinator relationships using a combination of field observation, breeding systems experiments, and comparative phylogenetics. A further study develops a molecular phylogeny for the 38 recognised genera of the epacrids, and the final study places the ecological pollination research into this evolutionary context.

Epacrid floral traits were related to bird, bee, and fly pollination systems. Bird pollination occurs in the most basal tribe, but insect pollination is also predicted for this tribe. Fly and bee pollination are widely supported by epacrid flowers, while red flowers and long floral tubes are the most important predictors for those visited by birds. Insect pollination is widespread across the evolutionary tree, but bird pollination coincides largely with the convergent evolution of red flowers. Purple, green, yellow, and nectarless flowers are distributed across the tree, also consistent with a hypothesis of convergent evolution. Nectarless flowers tend to be associated with wind or buzz pollination. Nocturnal mammals and lizards forage on epacrids but their role in pollination remains uncertain. Overall, the data suggest that pollinators have played a major role in the morphological diversification of the epacrids.

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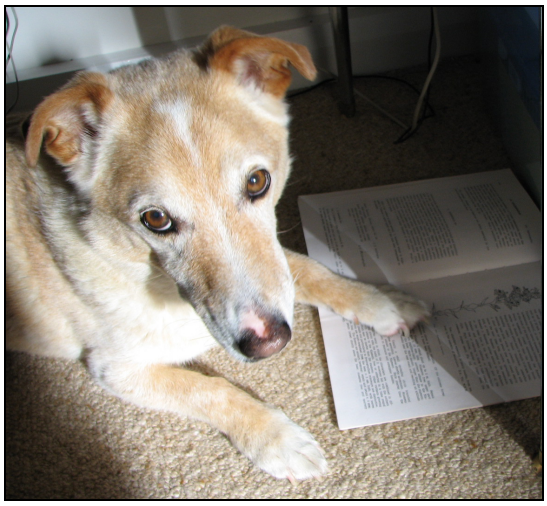

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Those unconcerned about the natural world, and I hope their numbers are dwindling by persuasion, will do well to consider the consequences for humanity of the decline of pollinator complexes. Eighty percent of the species of our food plants worldwide, we are informed, depend on pollination by animals, almost all of which are insects. One of every three mouthfuls of food we eat, and of the beverages we drink, are delivered to us roundabout by a volant bestiary of pollinators.

— Edward O. Wilson p.xiv Foreward to Buchmann SL and Nabhan GP (1996) The Forgotten Pollinators. Island Press, Washington, DC

Chapter 1 Introduction and aim

1.1 Introduction

Since the time of Sprengel and Darwin scientists have endeavoured to understand the processes that contribute to the huge numbers of angiosperm species and the morphological variation within them. Certain plant families have speciated dramatically and display a bewildering array of floral morphologies and associations with pollen vectors. The Orchidaceae are perhaps the most pre-eminent example – Charles Darwin (1862, 1877) proposed that differences in orchid floral features acquired since divergence from the common ancestor were adaptations to improve reproductive success through cross pollination. By the late 1700s and early 1800s, the concept of evolutionary descent with modification was already engaging scientists including Jean-Baptiste Lamarck and Erasmus Darwin (Avise, 2006). Charles Darwin added the concept of natural selection as the main driver of adaptive evolution (Darwin, 1859) and in so doing, influenced all modern work on pollination ecology and the evolution of pollination systems.

Speciose groups such as the orchids continue to inspire intense research efforts (Nilsson, 1992; Johnson et al., 1998; Schiestl et al., 1999; Garcia-Cruz and Sosa, 2005; Pauw, 2006; Fay and Chase, 2009; Micheneau et al., 2009). Other plant families have also proved to be fertile ground for developing ideas on plant-pollinator relationships and patterns and processes of evolutionary diversification. The South African Iridaceae is an examplar of convergent floral evolution and specialisation to particular pollinators or functional groups of pollinators (Goldblatt and Manning, 2006). Microevolutionary studies in this flora have found that shifts between pollinators may occur when a plant species coincides with a new pollinator; and macroevolutionary studies have found that shifting between pollinators is associated with diversification (Johnson, 2010). While pollination studies in the Orchidaceae and Iridaceae are well advanced, similar research in the Ericaceae has yet to gain momentum.

The Ericaceae is a conspicuous and important part of the flora across much of the globe (Stephens, 2004). Spanning temperate, warm temperate and montane tropical regions it consists of some 124 genera and 4100 species in eight subfamilies (Stephens, 2004). Pollination and floral visitor studies have been undertaken for parts of this extensive family

(Wallace, 1977; Dorr, 1981; Cane et al., 1985; Rathcke and Real, 1993; Jacquemart and Thompson, 1996; Navarro, 2001; Mejías et al., 2002; Cane and Schiffhauer, 2003; Navarro, 2006; Stout et al., 2006; Escaravage and Wagner, 2008; Navarro et al., 2008). The epacrids (subfamily Styphelioideae Sweet.) were formerly accorded family status as Epacridaceae, however, recent phylogenetic studies (Crayn and Quinn, 2000; Kron et al., 2002) revealed them to be a well-supported monophyletic group within the Ericaceae, where they are now recognised as a distinct subfamily (Stephens, 2004).

The epacrids comprise more than 550 species of woody plants ranging from small prostrate shrubs to temperate rainforest emergents (Stephens, 2004). Their geographical range extends from SE Asia eastwards through Oceania to Tierra del Fuego, but most of the species and the taxonomic diversity in the subfamily is found in Australia, particularly in the southwest and southeast; with other centres of diversity occurring in New Zealand and New Caledonia (Stephens, 2004). Although all epacrids have radially symmetrical flowers there is much floral variety, from the white cup-shaped and glabrous *Monotoca* to red tubular and hairy *Astroloma* flowers; from *Richea* species that abscise their corollas to present a brush-like flower to the small green and hidden flowers of *Acrotriche* (Curtis, 1963; Walsh and Entwisle, 1996).

Pollination research on epacrids has been largely descriptive. Biologists and naturalists have documented many epacrid flower visitors (Fletcher, 1977; Paton and Ford, 1977; Ford et al., 1979; Keighery, 1980; Thomas, 1980; Hawkeswood, 1993; Green and Osborne, 1994; Corbett, 1995; Keighery, 1996; Higham and McQuillan, 2000; Hingston and McQuillan, 2000; Houston, 2000; Hawkeswood, 2002; Houston and Ladd, 2002; Celebrezze and Paton, 2004; Hawkeswood and Turner, 2004; Schneemilch et al., 2011). Researchers have used information on floral morphology and the traditional pollination syndromes (Faegri and van de Pijl, 1979) for theoretical analyses of pollinators. Other researchers have targeted specific epacrids, and a few have undertaken plant breeding systems and selective animal exclusion experiments (Higham and McQuillan, 2000; Celebrezze and Paton, 2004). There has been no subfamily-wide approach to exploring the pollination ecology of epacrids, yet previous research provides the basis from which this can happen.

Since Darwin, flower adaptation to biotic pollinators has been offered as an explanation for floral diversity (Grant, 1949; Stebbins, 1970; Faegri and van de Pijl, 1979; Fenster et al.,

2004; Muchhala, 2006; Whitall et al., 2006; Fenster and Martén-Rodríguez, 2007; Streisfeld and Kohn, 2007; Kulbaba and Worley, 2008; Smith et al., 2008). It suggests that variations in traits among closely related species are adaptations to different pollinators, while floral convergence indicates adaptation to the same pollinators. The concept of floral convergence has in turn led to the identification of suites of floral characters (or pollination syndromes) associated with different types of pollinators (Faegri and van de Pijl, 1979). There are some floral characters that might be more readily influenced by pollinators than others, including changes in colour. Well-characterised model plant taxa (including species of *Nicotiana*, *Lotus*, *Petunia*, *Solanum*) are being used to understand the potential influences of animal pollinators on floral diversity through both the heritability of floral attributes and their levels of plasticity (Whitney and Glover, 2007). For instance, while flower size within a species is constrained by complex sets of interacting genes, flower colour is extremely labile (Whitney and Glover, 2007). Furthermore, neurobiologists have had success in linking the adaptive significance of flower signal evolution within species with pollinator sensory processes and cognition (Giurfa and Lehrer, 2001; Chittka and Raine, 2006).

The concept of pollination syndromes, whereby specific floral characteristics are associated with pollinator-type, dates back to Frederico Delpino (1833-1905) and is widely recognised through the work of Faegri et al. (1979). Pollination ecologists have observed syndromes that have arisen independently in distantly related plant lineages in association with particular types of pollination systems – both biotic and abiotic. Attracting specific animals to utilise as pollen vectors has been related to plant characteristics including flower morphology, colour, nectar, and fragrance (Faegri and van de Pijl, 1979; Herrera and Pellmyr, 2002; Fenster et al., 2004; Schaefer et al., 2004). For example, hummingbirds have been shown to be critical for the reproductive success of some plants with large, red, tubular flowers (Armesto et al., 1996). To attract hummingbirds the flowers must be conspicuous, including a colour that suits the birds' vision, and contain nectar (Cronk and Ojeda, 2008). Once the bird is attracted to the flower, the corolla shape directs the way in which it may have access. This provides an opportunity for a variety of co-adaptations such as reciprocally curved bills and curved tubular corollas; and the opportunity for pollen to be placed onto certain parts of the body of the bird (Temeles and Kress, 2003). Despite the potential for coevolution between flowers and pollinators, flowers range from the euphilic, restricting access to highly specialised and coevolved pollinators, to the allophilic, visited and influenced by many pollinators (Fenster et

al., 2004; Waser and Ollerton, 2006). Although the concept of pollination syndromes has provided a major framework for exploring how plants and pollinators interact, their utility for inference has been widely debated (Hingston and McQuillan, 2000; Ollerton et al., 2009).

Functional groups of animals (such as long-tongued bees, short-tongued bees, other Hymenoptera, Diptera, Coleoptera, Lepidoptera, Hemiptera, and birds) better assist our understanding of the evolutionary relevance of pollination syndromes than basic species lists (Fenster et al., 2004). A functional group of animals is made up of taxa that behave in similar ways on a flower and thus should exert similar selection pressures (Fenster et al., 2004). Correlations among floral traits may be the result of these pressures (e.g. the large, red, tubular corollas of the hummingbird-pollinated flowers) (Waser et al., 1996; Cronk and Ojeda, 2008). Although a plant may be visited by several functional groups of pollinators the relative selective pressures exerted are likely to be different for each group (Fenster et al. 2004). Stebbins (1970) suggested that the characteristics of a flower are formed by pollinators that visit it frequently and effectively. To explore effective functional groups of pollinators requires a quantitative approach such as frequency counts of 'legitimate' (contacting anthers and stigma and moving between conspecific plants) flower visitors (Ollerton et al. 2009), and especially breeding systems and selective animal exclusion experiments (Kearns and Inouye, 1993; Higham and McQuillan, 2000; Celebrezze and Paton, 2004).

Microevolutionary studies have assisted our understanding of single plant and pollinator relationships, but macroevolutionary studies that use a comparative approach such as mapping onto a phylogeny give insight into the frequency and direction of pollinator shifts. The latter part of the twenthieth century saw a new and powerful way to undertake comparative phylogenetics. DNA- and protein-level characters can now be used as a basis for phylogenetic comparisons with organism-level traits (Avise, 2006). As a result, scientists can employ independently estimated phylogenies, rather than those based for example, on floral morphology. This enables circularity to be minimised and sets up an environment for robust testing of the relationships between floral trait and pollinator evolution across the tree (DeWitt, 2010). Modern phylogenetic methods not only make it possible to infer how many times a particular trait has arisen but allow us to examine how floral traits respond to changes in pollinator and how these transitions affect the fates of entire plant lineages (DeWitt, 2010). Although we are continually discovering that many biotic and abiotic forces are involved in

shaping floral traits, there is mounting evidence to suggest that pollinators play a key role (Schemske and Bradshaw, 1999).

1.2 Knowledge gap

There has been no comprehensive study that explores the ecological and evolutionary relationships between floral traits and pollinators across the iconic Styphelioideae. The speciose nature of the subfamily, obvious floral variation, and geographic occurrence of most species and taxonomic diversity in Australia make it an ideal candidate for such research. Although Australian researchers and naturalists have a history of recording associations between epacrids and their floral visitors (refer Appendix 7-2) there have been relatively few in depth studies of the pollination ecology (including the examination of floral trait and pollinator profiles, and/or breeding system experiments) of individual species or genera. The most comprehensive works in this respect are Ladd (2006), Celebrezze and Paton (2004), Houston and Ladd (2002), Higham and McQuillan (2000), and Schneemilch and Steggles (2010).

Separating floral trait adaptation from historical effects is critical for our understanding of how pollinators may influence floral evolution. Robust molecular phylogenies provide a context for interpreting the evolution of plant and animal lifestyles and morphologies at a level removed from individual species. They make it possible to infer how many times a floral trait has arisen, and provide a means of testing broader theories such as those on the relationships of floral traits to pollination systems. Despite a rich history of pollination studies in the angiosperms, the application of phylogenetic approaches to pollinator-mediated floral evolution is still in its infancy (DeWitt, 2010). Evolutionary relationships among taxa within the Styphelioideae have been recently explored (Crayn et al., 1998; Quinn et al., 2003; Quinn et al., 2005) but no subfamily-wide evolutionary hypothesis has previously been produced. Thus, until now, there has been no framework to support a comparative phylogenetic approach to examining the association between floral features and pollinators across the subfamily.

By employing independently estimated phylogenies rather than those based on floral morphology, and by observing pollination systems through field studies rather than inference from floral morphology, it is possible to robustly test relationships between pollinators and

floral trait evolution (DeWitt, 2010). Relatively few studies have used comparative methods to explore how pollinators affect floral evolution across the phylogeny and studies that estimate correlations among traits and pollinators remain rare (DeWitt, 2010). With the exception of Houston and Ladd (2002) who mapped the occurrence of buzz pollination onto a phylogeny by Powell et al. (1997), there has been no such work undertaken for the Styphelioideae.

We cannot expect a perfect correspondence in the adaptation of floral traits to pollinators: a trait may arise by genetic drift (nonadaptation), as a result of other selective forces (e.g. herbivory) or some lineages may not have the capacity for a particular trait (DeWitt, 2010). Thus, we need to test the validity of hypotheses about relationships between floral features and pollinators. Currently, there is a gap between what is possible and what has been done in regard to statistically testing these relationships. No previous study has gone beyond testing the relationships between single epacrids and their pollinators.

No phenomenon illustrates more vividly than pollination ecology the need for conservation measures to be driven by an understanding of plant-animal interactions. Research in this area will assist us to move beyond the paradigm of single species conservation to management that addresses plant reproduction and animal foraging requirements. Tasmania has a rich endowment of epacrid species many of which are endemic to the island (Baker and Duretto, 2011). Along with the south west of Western Australia, Tasmania is one of the epacrid hotspots of the world, making it an ideal place to undertake such research.

1.3 Aim of thesis

The aim of the thesis is to determine the relationships between epacrid floral features and potential pollinators, and outline the evolutionary status of any pollination syndromes. The main hypotheses tested were that:

1. flower characteristics relate to pollinators in predictable ways
2. there is convergent evolution in the development of pollination syndromes

1.4 Thesis Overview

My thesis is a collection of research papers, either published (Ch 2-4), submitted for publication (Ch 6), or in preparation for submission (Ch 5 & 7). Each of chapters 2-7 constitutes a stand-alone paper. No changes have been made to the contents of the previously published papers presented here. However, for ease of reading I have standardised (with the exception of the references) their formats. Acknowledgements, references, appendices, and supplementary material are included at the end of each chapter. While each of these chapters may be read in isolation from the others, in combination they address the thesis aim stated above by providing an overview of the pollination ecology and evolutionary relationships of the Styphelioideae. The chapters are as follows:

Chapter 2 tests the hypothesis that one of the most basal epacrids, *Prionotes cerinthoides* is bird-pollinated. It has been postulated that red and pink tubular flowers are indicative of bird pollination and *Prionotes* has such flowers. I undertake an in depth study of the breeding system and effective pollinators.

Chapter 3 is devoted to the enigmatic *Acrotriche serrulata.* Given its cryptic habit and unusual flowering, including secondary pollen presentation, it has long been suspected that *Acrotriche* requires very specific pollinator behaviour to effect pollination. I study the breeding system, and explore the potential for insects, lizards and mammals to act as pollinators.

Chapter 4 tests the hypothesis that the flowers of *Sprengelia incarnata* and *S. propinqua* are sonicated by native bees. *Sprengelia incarnata* has been suggested as a candidate for sonication because of its nectarless flowers and stamen morphology. *Sprengelia propinqua* shares many similar features. I examine bee behaviour, floral morphology, pollen tackiness, and document potential pollinators.

Chapter 5 tests whether the evolution of floral diversity in *Richea* species relates to pollinator profiles. *Richea* species exhibit an uncommon form of flowering where the corolla forms an operculum which does not open. Instead, it is shed to expose the reproductive organs. Here I determine their biotic pollinators, assess the relationships between floral trait profiles and pollinator profiles, and examine floristic diversity in two divergent evolutionary groups.

Chapter 6 is devoted to producing a genus level phylogeny of the Styphelioideae. Published sequence datasets of five markers are now available for all except one of the 38 recognised genera. However, several markers are highly incomplete therefore missing data is problematic for producing a phylogeny. I explore the relative utility of supertree and supermatrix approaches for addressing this challenge, and examine the effects of missing data on tree topology and resolution.

Chapter 7 explores whether there is convergent evolution in the development of pollination syndromes, and explores the ecological and evolutionary relationships between epacrid floral traits and pollinator profiles. I establish and test specific pollination syndromes for the Styphelioideae from the known plant and pollinator relationships and use them to infer pollination systems where they are currently unknown. I use the Styphelioideae phylogeny from Chapter 6 as the backbone upon which to provide a hypothesis of the evolutionary history of pollination systems.

1.5 Contributions of Co-authors

I have been responsible for the majority of ideas, project design, implementation, field work, data analyses, and writing of the papers presented. However, my work has benefited from the contributions of the co-authors named at the start of Chapters 2-6. The topic for my thesis was developed in conjunction with my supervisors Dr. Peter McQuillan and Prof. Jamie Kirkpatrick.

For Chapter 2 Dr. Peter McQuillan provided advice on methodology, contributed a day of field work to assist in the establishment of the breeding system experiments, provided expertise on invertebrate identification, had input into the analysis of the invertebrate data, and a minor role in editing. Prof. Jamie Kirkpatrick provided advice on data analysis, had input into the structuring of the paper, and was the main editor. I undertook most of the field work, data collection and analyses, and wrote the manuscript.

For Chapter 3 Dr. Peter McQuillan contributed half a day in the field and half a day in the lab to assist in invertebrate identification. Prof. Jamie Kirkpatrick provided advice on data analysis and was the main editor. I undertook most of the field work, data collection and

analyses, and wrote the manuscript.

For Chapter 4 Dr. Peter McQuillan contributed a day in the field and assisted with invertebrate identification. I undertook most of the field work, data collection and analyses, and wrote the manuscript.

For Chapter 5 Prof. Jamie Kirkpatrick provided advice on data analyses and was the main editor. I undertook all of the field work, data collection and analyses, and wrote the manuscript.

For Chapter 6 I approached experts in the field of phylogenetics for assistance. Thus, a substantial contribution to the chapter was made by Dr. Barbara Holland and this is recognised in her status as equal author. Barbara contributed to all aspects of the chapter, in particular she undertook the phylogenetic analyses. Prof. Darren Crayn assisted in obtaining some of the sequence data, and the manuscript benefited from his editorial role. Margaret Heslewood provided some unpublished sequences and commented on a draft. My contribution to this chapter was the concept to produce a genus-level hypothesis of the Styphelioideae; the determining and bringing together of collaborators; obtaining most of the sequence data from GenBank and elsewhere; and writing most of the manuscript.

Contributions made by other people can be found in the acknowledgements section at the end of each chapter.

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Chapter 2 Bird Pollination of the climbing heath *Prionotes cerinthoides*

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2.1 Abstract

Tubular, red and pink flowers often indicate bird pollination. *Prionotes cerinthoides*, a climbing shrub of temperate rainforest in Tasmania (Australia), and one of only two members of the most primitive clade of the subfamily Styphelioideae (Ericaceae), has such flowers. We tested the hypothesis that *P. cerinthoides* is bird-pollinated using breeding systems experiments, flower visitor observations and invertebrate trapping. Flowering phenology, nectar availability and flower damage were also recorded. *P. cerinthoides* produced little viable seed in the absence of a pollinator but selfed readily when pollination was facilitated. It appears that *P. cerinthoides* depends largely on the pollination services of a single native bird species, the eastern spinebill (*Acanthorhynchus tenuirostris*). The only other flower visitor observed to contact anthers and stigma was the introduced bumblebee (*Bombus terrestris*). The crescent honeyeater (*Phylidonyris pyrrhoptera*), introduced honeybee (*Apis mellifera*) and bumblebee were nectar-robbers.

2.2 Introduction

An understanding of pollination ecology and breeding systems is critical in the conservation management of vascular plant species (Buchmann and Nabham, 1996; Koptur, 2006; Kwak and Bekker, 2006). Particular morphological features of flowers have been suggested as associated with particular pollination systems (Faegri and van der Pijl, 1979), although the correspondence can be unreliable (Hingston and McQuillan, 2000; Robertson et al., 2005). Red and pink tubular flowers have been thought to be indicative of bird-pollination (Faegri and van der Pijl, 1979). There are many data to support this proposition, but the occasional exception. For example, in temperate rainforest in southern Chile, one hummingbird, *Sephanoides sephaniodes* Molina*,* was recorded as the main visitor to 14 red or pink flowered plants spanning a wide range of life-forms, including vines, epiphytes, hemiparasites, shrubs and trees (Armesto et al., 1996), while the South American scarlet-red, long-tubular flowered *Ourisia poeppigii* Benth.*,* despite its ornithophilous phenotype, is highly self-compatible and strongly autogamous (Arroyo and Penaloza, 1990). The morphological and phenological features of flowers are thought to impose constraints on the modes of pollination (Lloyd and Schoen, 1992). Many epacrids have both protandry and well-separated anthers and stigmas (Keighery, 1996).

The unusual climbing heath, *Prionotes cerinthoides* (Labill.) R.Br., is endemic to temperate rainforest, subalpine and alpine plant communities in Tasmania, Australia. *Prionotes* is a member of the Prionoteae, the most basal tribe of the epacrids, the other member being the monotypic South American genus *Lebetanthus* Endl. (Stephens, 2004). *Prionotes cerinthoides* is a slender perennial climbing or epiphytic shrub that ascends tree trunks, particularly of *Nothofagus cunninghamii* (Hook.) Oerst.*,* and clambers over rocks in the alpine zone. The flowers are solitary, 20-25 mm long, crimson pink, tubular and pendulous on slender pedicels at the ends of leafy branches (Curtis, 1963). The flowers have an autumn anthesis when insect activity is declining in Tasmania. These attributes suggest that *P. cerinthoides* might be bird-pollinated. However, it is not possible to rule out a role for autogamy in this hermaphroditic species, as its anthers and stigma are positioned close together at the corolla mouth. Nor is it possible to rule out insect pollination, as the apparently ornithophilous New Zealand mistletoes were found to be pollinated by birds and insects (Robertson et al., 2005). *Prionotes cerinthoides* appears to produce little viable seed and may be pollinator-limited

(Lawrence, 1992). We used observations and experiments on the breeding system, flowering phenology, flower visitors, flower damage and nectar production of *P. cerinthoides* to determine whether it is bird-pollinated.

2.3 Methods

2.3.1 Study site

The study site was located in the Tasmanian Wilderness World Heritage Area ~ 70 km west of Hobart (42º49'55"S 146º23'06"E) and 560 m asl on Precambrian quartzite. The rainfall at nearby Strathgordon is 2536 mm per year, spread over at least 200 days with the wettest month being August and the driest February (Bureau of Meteorology, 2008). February is also the warmest month averaging a daily maximum of 20 C and a daily minimum of 10 C, and July is the coldest averaging a daily maximum of 9 C and a daily minimum of 3 C (Bureau of Meteorology, 2008). Experiments and observations were carried out within an area of about 1.5 hectares set within a much larger area of thamnic rainforest (Jarman et al., 1984). The most common vascular plants in the study area were the trees *Nothofagus cunninghamii*, *Eucryphia lucida* and *Phyllocladus aspleniifolius* (Labill.) Rich. ex Hook.f.; the shrubs *Anodopetalum biglandulosum* (A.Cunn. ex Hook.) Hook.f., *Richea pandanifolia* subsp. *pandanifolia* Hook.f., *R. milliganii* (Hook.f.) F.Muell., *Trochocarpa gunnii* (Hook.f.) Benth., *T. cunninghamii* (DC.), *Anopterus glandulosus* Labill., *Tasmannia lanceolata* (Poir.) A.C.Smith, *Tetracarpaea tasmanica* Hook.f. and *Olearia tasmanica* W.M.Curtis; the tall sedge *Gahnia grandis* (Labill.) S.T.Blake, and the ferns *Hymenophyllum peltatum* (Poir.) Desv., *Grammitis billardierei* Willd. and *Blechnum wattsii* Tind. There was a rich bryophyte flora on the ground and tree trunks. Vascular plant nomenclature follows Buchanan (2007).

2.3.2 Breeding system experiments

Fifty *P. cerinthoides* plants were randomly selected and tagged. For the breeding system experiment, each plant was allocated to one of four pollination treatments: autogamous selfing, hand-selfing, hand-crossing, bird-exclusion or open control. A minimum of 50 flower buds (10 buds from each plant and mostly accessible from the ground) were assigned to each treatment with more used if available to compensate for anticipated losses to herbivory.

Before bagging, stems were checked for phytophagous insects and any found were physically removed. For the bird-exclusion experiment, cages and open controls were located on the same plants. Crescent honeyeaters (*Phylidonyris pyrrhoptera* (Latham)) were observed foraging near cages and did not appear repelled by them. The treatments were as follows:

2.3.2.1 Autogamous selfing

A total of 57 unopened buds distributed across five plants were bagged with Terylene mesh bags (~ 20 cm by 30 cm, with ¼ mm mesh) to prevent animal visitors. No other manipulation was undertaken.

2.3.2.2 Hand-selfing

A total of 55 unopened buds across five plants were opened and emasculated using tweezers and pollinated from another flower on the same plant. As pollen is shed before buds open, only buds with intact pollinia were chosen for emasculation - pollen was found to dehisce prior to buds opening. Buds were opened with tweezers for placement of pollen onto stigmas. A toothpick was used to transfer pollen from one flower to another. Hand pollination was carried out once, immediately after emasculation. The presence of pollen grains (~ 30 - 40 μm in diameter) on stigma was checked with a hand lens. Following treatment, terylene mesh bags (as for autogamous selfing) were used to cover the flowers.

2.3.2.3 Hand-crossing

A total of 53 unopened buds across five plants were opened and emasculated using tweezers and pollinated (as for hand-selfing) from a flower on a *P. cerinthoides* plant growing at least five metres away.

2.3.2.4 Breeding system open control

A total of 66 buds across five plants were marked with green twist ties and left open to natural pollinators. Twist ties were located on the same branch as the flower but distant from the flower to minimise any possible influence on flower visitors.

2.3.2.5 Bird-exclusion

A total of 116 buds from 15 plants were enclosed in wire cages (~ 20 cm by 12 cm, with 8 mm mesh) to exclude birds. The cages would also have excluded larger moths and butterflies. The cages would not have excluded honeybees which are known to pass through mesh as small as 5 mm (Hingston et al., 2004). It is possible that larger bumblebees may have been excluded by an 8 mm mesh. In southern Tasmania, Hingston et al. (2004) found no significant difference between the visitation rates of all insects, exotic bees, native bees, wasps, ants and beetles, within cages with mesh sizes of 5 to 25 mm.

2.3.2.6 Bird-exclusion open control

A total of 122 buds from 15 plants were marked with blue cotton thread and left open to all natural pollinators.

2.3.2.7 Capsule and seed set

Capsules were collected prior to dehiscence, wherever possible. Maturity was generally accompanied by an obvious colour change from green to brown, although this change was less obvious in capsules in the Terylene bags. This colour change was used as a signal to collect capsules for further analysis. For the breeding system experiment, capsule length, width and weight were measured. For each capsule, seed size (longest dimension) and the presence or absence of endosperm within seeds was determined by cutting the seed in half and examining it under a dissecting microscope. Seeds from each capsule were placed into size classes and the proportion in each class recorded. For confirmation of seed viability, over 600 seeds with endosperm (including all 15 from the autogamous selfing treatment) and over 600 seeds without obvious endosperm were placed in Petri dishes on filter paper and kept moist at room temperature for over four months to encourage germination. For the bird-exclusion experiment, capsule length, width and weight were measured.

2.3.3 Phenology

One hundred buds / flowers / fruits were collected in each month from February to May 2007. Each month, five locations at the site were chosen in an *ad hoc* fashion, with at least three metres between them. Within each sample twenty adjacent buds / flowers / fruits were

collected. Stage of development, corolla length, width at mouth of corolla, stamen length, pistil length and stigma diameter were recorded using a dissecting microscope.

2.3.4 Flower damage

To limit the numbers of flowers removed from the area, flower damage was assessed using a subset of the samples used for phenology (flowers and buds > 11.5 mm). The total varied between months as follows: Feb 59; Mar 71; Apr 26. Each flower or bud was scored for the presence of damage: none; bruise; hole. Where both a bruise and hole were present only the hole was noted. The sizes of bruises and holes were measured to the nearest 0.5 mm along their longest dimensions.

2.3.5 Flower visitors

Fifty-two hours of visitor observations were made on ~ 1400 flowers during peak flowering, between late February and late April 2007 (27-28 Feb, 1 Mar, 7 Mar, 9-10 Mar, 15 Mar, 22 Mar, 20 Apr). Observations took place on clear, windless and relatively warm days (including dawn and dusk) and five hours were undertaken over two mild nights. Flowers with pollen present were chosen for observation. The frequency of visitors, number of flowers visited and time spent at each flower was tabulated. Observations were undertaken in person and by video camera (Panasonic Digital Video Camera, model number NV-GS70, 1.7 mega pixel, 500 x digital zoom) set on a tripod. Flower visitors were defined as follows:

- potential pollinator - vector observed removing and / or depositing pollen (or where there was evidence that this may occur) and traveled between flowers of the same species (or evidence that this may occur)

- flower visitor - vector not observed removing and / or depositing pollen; may or may not be observed to travel between flowers of the same species.

Twenty sticky traps (Bar-Ness et al., 2006) each with an area of 336 cm^2, were used to examine the local availability of potential insect-pollinators. Traps were exposed in pairs with one facing towards and within 50 cm of a flowering *P. cerinthoides* plant, and one facing away from, and at least one metre distant of the plant. Traps were left in the field for 9 days during the peak flowering period (7-15 Mar 2007). Two traps were excluded from analysis. One trap had malfunctioned and its sticky surface was not exposed at the time of collection.

Another had adhered vertebrate animal fur suggesting that it may have been tampered with.

2.3.6 Nectar availability

The pattern of diurnal nectar availability was assessed using a microcapillary tube to measure nectar in the morning (0930 to 1100 hrs) and afternoon (1530 to 1730 hrs). As microcapillary tubes are unlikely to extract all nectar (Kearns and Inouye, 1993), this convenient field technique was used to gain an indication of relative nectar availability only. Flowers were chosen in an *ad hoc* fashion with the only requirement being that the anthers must still have pollen present and that there was no discernible pollen on the stigma. The surveys were as follows:

- Nectar was measured in the morning, flowers were bagged to exclude visitors and remeasured in the afternoon.
- Nectar was measured in the morning, flowers were left exposed (tagged with green twist ties) and remeasured in the afternoon.
- Nectar was measured in the morning, flowers removed and different flowers measured in the afternoon.
- Flowers were bagged by 8:00 am and nectar measured only in afternoon.

Surveys 1 to 3 were carried out on a fine, warm and dry day on 22 March 2007 and survey 4 on a fine, mild day after heavy dew on 24 March 2007; all surveys were carried out on 30 flowers.

2.3.7 Data analysis

The linear relationships between capsule height, width and weight were determined. The Andersen-Darling test was used to assess if data departed from a normal distribution. The null hypothesis that all capsule weights are taken from populations with the same median was tested using the Kruskal-Wallis H test. The Mann-Whitney U test was used to investigate the significance of differences in median capsule weight between breeding system and exclusion trials. The null hypothesis that capsule weight is not a predictor of the presence of viable seeds (defined as seeds with a length of ≥ 0.5 mm) was assessed using regression analysis. All

tests were carried out in MINITAB 15 with default settings. The breeding system open control and bird-exclusion open control were combined for analysis. Any flowers found near cage edges (and possibly accessible to birds) were not included in analysis. Eleven flowers from the hand-selfed treatment were removed from analysis, after being exposed to open pollination when the mesh bag was tampered with by animals.

The null hypothesis of no difference in the insect profile (species and numbers) between paired sticky traps was tested. The significance of any difference in the membership of the two groups of traps was determined using the multi-response permutation procedure (MRPP) following ordination based upon Bray-Curtis dissimilarity values in PC-ORD (McCune and Mefford, 1999). The difference between morning and afternoon nectar availability was analysed using Student's paired t-test when revisiting the same flower and Student's two sample t-test when the samples were independent.

2.4 Results

2.4.1 Breeding system, capsule and seed set

All flowers across all treatments produced capsules except those that aborted in the bud stage. Capsule variables were highly inter-correlated (weight and height $R^2 = 0.80$, $P = < 0.001$; weight and width $R^2 = 0.81$, $P = < 0.001$; height and width $R^2 = 0.77$, $P = < 0.001$), so capsule weight data were used in further analysis. Capsule weight was not normally distributed (Andersen-Darling test: Mean 0.008, sd 0.006, n 286, AD 15.388, $P < 0.005$). The null hypothesis that all capsule weights are taken from populations with the same median was rejected (Kruskal-Wallis test: H = 122.71, df = 4, $P < 0.001$ adjusted for ties).

Capsules produced as a result of the autogamous selfing and bird-exclusion treatments were generally small, usually did not open and rarely had one or two seeds containing endosperm. In contrast, the hand-selfing and hand-crossing pollination treatments usually had a mix of ovules and seeds. The capsules opened and the seeds were dehisced while the ovules remained attached to the placenta. The largest and heaviest capsules (weight 0.013 ± 0.004 g) were produced in the hand-crossing pollination treatment and the smallest and lightest

capsules (weight 0.004 ± 0.001 g) in the autogamous selfing treatment (Fig. 2-1). Breeding system treatment was found to be a very significant factor in determining capsule weight with most treatments significant at $P < 0.001$ (Table 2-1). Open control and bird-exclusion were significant at $P < 0.01$. Autogamous selfing and bird-exclusion, and hand-selfing and hand-crossing were significant at $P < 0.05$.

Capsules containing viable seed were generally easily distinguished by eye as were seeds that would germinate. The majority of seeds with endosperm grew, including all seeds (n = 15) from the autogamous selfing trial. *Prionotes cerinthoides* is capable of autogamous selfing but produces much lower numbers of viable seed by this method than by hand-crossing. Generally some viable seeds are present when a capsule weighs over 6 mg and capsule weight is a predictor of the percentage of viable seeds in a capsule ($R^2 = 0.672$, $P < 0.001$). The length of unviable ovules ranged from < 0.2 mm to 0.5 mm while viable seeds were always > 0.5 mm and mostly > 1.2 mm. Viable seeds were found in 96% of capsules in the hand-crossing treatment and 95% in the hand-selfing treatment compared with 4% of capsules in the autogamous selfing treatment. Thirty-nine percent of the control capsules contained viable seed. It was estimated that capsules generally contained over 100 unviable and / or viable seeds (the highest number counted was 160). Counting was complicated by dehiscence of viable seeds and the small size of the unviable ovules. Many capsules, particularly from the autogamous selfing treatment, contained 100% unviable seeds, but no capsules, even from the hand-crossing pollination treatment, contained 100% viable seeds.

Figure 2-1 Capsule mass by breeding system treatments. Note: box = 50% of observations, whiskers = rest of observations, line = median

Table 2-1 Significance of differences in median capsule mass (mg) between treatments (Mann-Whitney *U* -test)

	Treatment 1		Treatment 2			
Treatment (1 vs. 2)	*n*	Median 1	*n*	Median 2	*W*	*P*
Autogamous selfing v hand-crossing	48	4	50	13	1220.5	< 0.0001
Autogamous selfing v open control	48	4	94	5	2369.5	< 0.0001
Autogamous selfing v bird-exclusion	48	4	49	4	2055.0	0.0324
Autogamous selfing v hand-selfing	48	4	45	11	1234.5	< 0.0001
Hand-selfing v hand-crossing	45	11	50	13	2707.5	0.0221
Hand-selfing v open control	45	11	94	5	5713.0	0.0001
Hand-selfing v bird-exclusion	45	11	49	4	1403.5	< 0.0001
Hand-crossing v open control	50	13	94	5	4929.5	< 0.0001
Hand-crossing v bird-exclusion	50	13	49	4	3598.0	< 0.0001
Open control v bird-exclusion	94	5	49	4	7480.5	0.0020

2.4.2 Phenology

Anthesis commenced in late February and was largely finished by May (Fig. 2-2), although an occasional flower was still observed as late as September. From late March until early spring, *P. cerinthoides* was the only species in flower at the study site. Flowers had the following average dimensions: corolla length 23.5 ± 2 mm, corolla width 5.9 ± 1.0 mm, stamen length 23.5 ± 2.1 mm, pistil length 24.8 ± 2.2 mm and stigma width 0.9 ± 0.2 mm. The anthers were level with the corolla mouth and the pistil was generally extended about 1 mm beyond (Fig. 2-3). Most subsequent fruits matured (turned brown) in November and December.

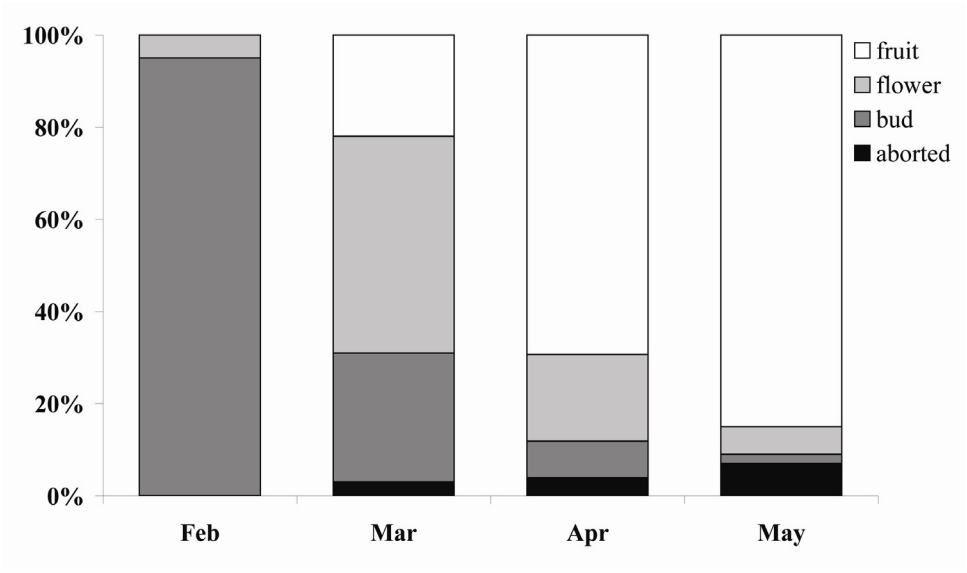

Figure 2-2 Flowering and fruiting phenology in *P. cerinthoides*

Figure 2-3 *P. cerinthoides* showing position of anthers and style; sheltering erythraeid mite

2.4.3 Flower damage

Herbivory and other pressures progressively depleted the numbers of capsules produced in the experiments (Table 2-2). The unbagged treatments showed a lower survival rate through to capsule production than the bagged treatments. The number of flowers damaged and the extent of the damage increased from February to April (Figs. 2-4 to 2-6). Both insects and birds were responsible for damage to flowers. The crescent honeyeater (*Phylidonyris pyrrhoptera*), bumblebee (*Bombus terrestris* (L.)) and honeybee (*Apis millifera* L.) were observed to make holes in flowers for the purpose of nectar-robbing and an endemic parrot, the green rosella (*Platycercus caledonicus* (Gmelin)), completely destroyed flowers by shredding them. A crescent honeyeater was observed to use an existing hole at the base of a *P. cerinthoides* flower for nectar-robbing.

Table 2-2 Transition from buds to fruit in bagged and open treatments. Note: * 11 flowers removed from experiment after exposure to open pollination. Survivorship of remaining flowers is shown.

Treatment	Number of buds at start of experiment	Number of fruits at end of experiment	Survival (%)
Hand-crossing (bagged)	53	53	100
Hand-selfing (bagged)	44*	44	100
Autogamous selfing (bagged)	57	56	98
Open control for breeding system	66	52	79
Open control for bird-exclusion	122	62	51

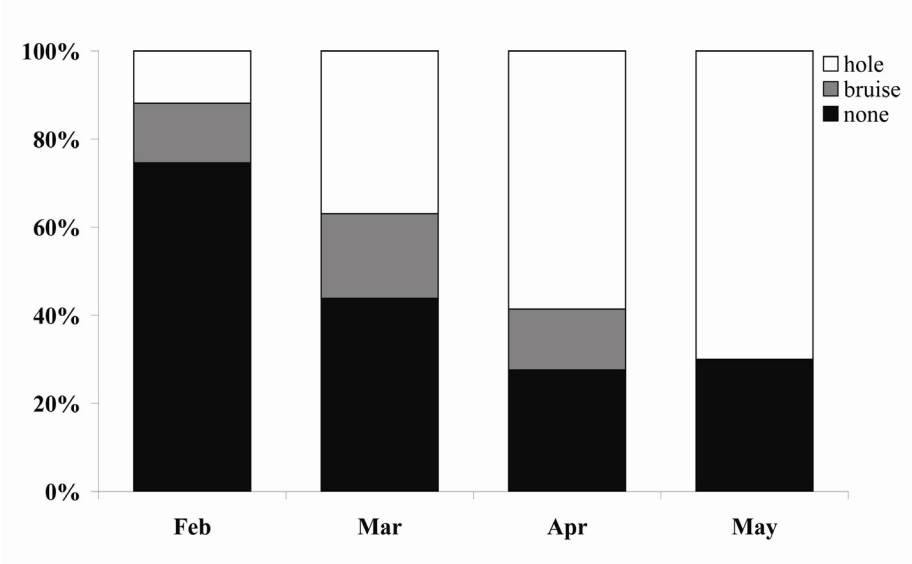

Figure 2-4 Damage to corollas of *P. cerinthoides* over the flowering period

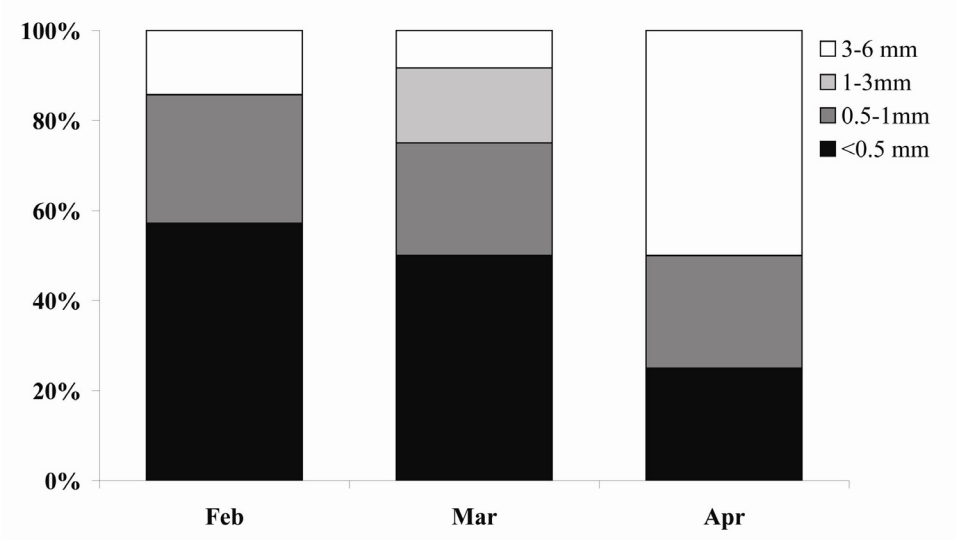

Figure 2-5 Profile of bruise damage in *P. cerinthoides* flowers

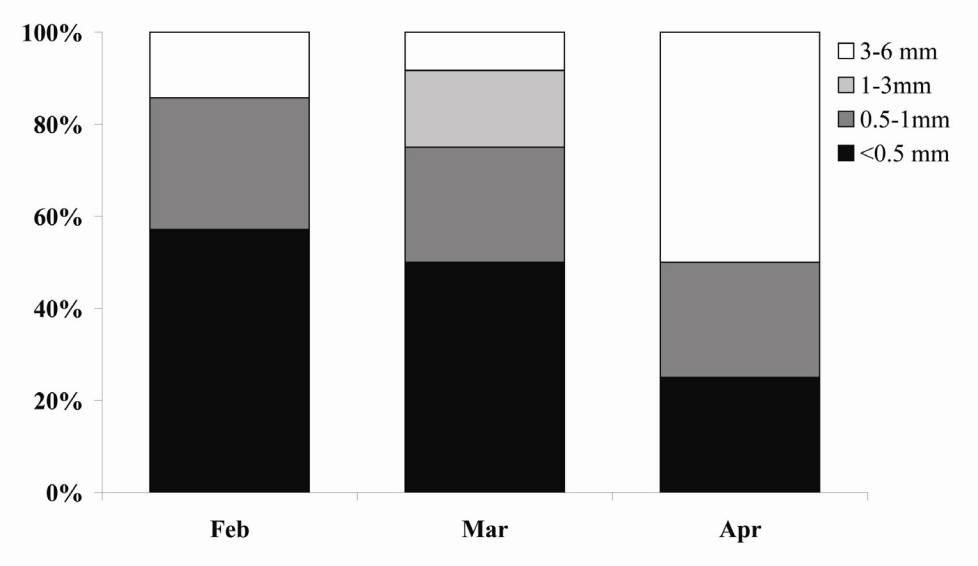

Figure 2-6 Profile of hole damage in *P. cerinthoides* flowers

2.4.4 Flower visitors

The eastern spinebill (*Acanthorhynchus tenuirostris* (Latham)) and introduced bumblebee (*Bombus terrestris*) were the only potential pollinators observed. The visitation rate was extremely low with eight individual eastern spinebills and five bumblebees observed to visit flowers in 52 hours of observations (Table 2-3). The eastern spinebill was the only native animal observed to visit flowers in a manner consistent with a potential pollinator. The introduced bumblebee was observed, on one occasion, to contact anthers and stigma as it gathered pollen but was more usually a nectar-robber. *Prionotes cerinthoides* pollen was observed on the bumblebee. The crescent honeyeater is a known pollinator of various plant species, including the Tasmanian epacrid, *Leptecophylla divaricata* (Hook.f.) C.M.Weiller (Higham and McQuillan 2000) but was observed to visit *P. cerinthoides* as a nectar-robber, accessing the flower by piercing a hole at the base of the corolla tube. One crescent honeyeater was observed to use a pre-existing hole that was already present at the base of the corolla. Honey bees (*Apis mellifera*) also acted as nectar-robbers. A native ant (*Camponotus* Mayr.) was observed inside a flower at the nectaries and is likely to constitute a nectar-robber. Ants, spiders (family Araneidae and the common flower spider, *Diaea* Thorell) and spider mites (family Erythraeidae) appeared to be anthophiles but ineffective as pollinators. They were not observed to move between flowers and were not observed to contact anthers and

stigma, although the proximity of the anthers and stigma to the corolla mouth makes it possible that they could. With the exception of ants, these animals are not known as pollinators.

The most diverse and numerous insects caught on sticky traps were small flies (order Diptera) of which the Phoridae were the most prominent of the ten families recorded (Appendix 2-1). The second most common order was Coleoptera (beetles) represented by four families of which rove beetles (family Staphylinidae) were the most widespread across the traps. There was no significant difference in the communities of insects captured by traps placed near flowering *P. cerinthoides* plants and traps placed away from them than would be expected by chance (chance corrected within group agreement, A = - 0.002, delta obs. = 0.521, P = 0.421). Of the insects observed, blow flies (family Calliphoridae) are known to act as pollinators of plants. However, this taxon was not observed visiting *P. cerinthoides* and no pollen was observed on the one specimen found on a sticky trap. A number of other visitors, including flies from the families Mycetophilidae and Cecidomyidae and beetles from the Staphylinidae, Scirtidae and Melandryidae, are known to pollinate native flora but are not regarded as generalist pollinators and have specific relationships with particular plants, for example fungus gnats (family Mycetophilidae) are pollinators of some orchids, particularly Pterostylis R.Br. Sticky traps are unlikely to retain robust insects above about 8 mm in body length (an 8 mm hoverfly (family Syrphidae) was present on a trap). Although present in the study area, no honeybees or bumblebees were captured on sticky traps.

Various moths were observed in the vicinity of *Prionotes* at dusk and during night observations. These were from the families Geometridae (*Conosara castanea* Meyrick, *Microdes* Guenée and *Poecilasthena*Warren) and Oecophoridae (two species of *Barea* Walker). Also observed were a caddisfly (order Trichoptera) from the family Leptoceridae, and crickets (order Orthoptera). None of these animals were observed to interact with *P. cerinthoides*.

Table 2-3 Flower visitors to 1400 *P. cerinthoides* flowers over 52 hours in February to April 2007. Note: * PP = potential pollinator, FV = flower visitor, D = destroyed flower(s), S = stayed at flower

Visitor	Visit type*	No. individuals observed	No. flowers visited during observation(s)	Average time per flower (sec)	Shortest time per flower (sec)	Longest time per flower (sec)
Birds						
Eastern spinebill *Acanthorhynchus tenuirostris*	PP	8	26+	4	1	10
Crescent honeyeater *Phylidonyris pyrrhoptera*	FV	4	16+	2	1	2
Golden whistler *Pachycephala pectoralis*	FV	1	2+	1	1	1
Green rosella *Platycercus caledonicus*	FV	1	20+	D	D	D
Insects						
Bumble bee *Bombus terrestris*	FV PP	5	15+	4	1	10
Honey bee *Apis mellifera*	FV	2	9	8	2	24
Mite Erythraeidae	FV	3	3	S	S	S
Ant *Camponotus* sp.	FV	1	1	S	S	S
Spiders						
Flower spider *Diaea* sp.	FV	2	2	S	S	S
Arachnids Araneidae	FV	1	1	S	S	S

Of the birds observed in the study area, only the eastern spinebill and crescent honeyeater are known to pollinate plants in the Tasmanian Ericaceae (Higham and McQuillan 2000). The other birds observed included the brown thornbill (*Acanthiza pusilla* (Shaw)), green rosella (*Platycercus caledonicus*), scrubtit (*Acanthornis magnus* (Gould)), brush bronzewing (*Phaps elegans* (Temminck)), black currawong (*Strepera fuliginosa* Gould), forest raven (*Corvus tasmanicus* Mathews), grey shrike-thrush (*Colluricincla harmonica* Latham), golden whistler (*Pachycephala pectoralis* Latham), pink robin (*Petroica rodinogaster* (Drapiez)) and white-browed scrub wren (*Sericornis frontalis* (Vigors & Horsfield)).

2.4.5 Nectar availability

Nectar availability was significantly less in the afternoon, regardless of whether flowers were bagged after nectar removal in the morning ($t = 3.75$, $P = 0.001$) (morning range 0-18 µl, average 3.1 µl; afternoon range 0-1 µl, average 0.2 µl) or marked with twist ties after nectar removal ($t = 3.84$, $P = 0.001$) (morning range 0-14 µl, average 2.5 µl; afternoon range 0-2 µl, average 0.3 µl). Nectar availability was also significantly less in the afternoon when flowers were removed after measuring and different flowers measured in the afternoon ($t = 3.39$, $P = 0.002$) (morning range 0-15 µl, average 2.3 µl; afternoon range 0-6.5 µl, average 0.4 µl). Generally, the only flowers that had nectar present in the afternoon were those that were bagged in the early morning, without nectar removal (range 1-16 µl; average 8.2 µl). Nectar was mostly present in flowers in the morning. The amount of nectar varied between flowers with some having much more than others. It is uncertain how much of this was actual variation and how much was the result of some flowers being visited by animals prior to sampling. The largest amount of nectar sampled from any flower was about 18 µl, although the amount present is likely to be dependent on the weather conditions.

2.5 Discussion

Prionotes cerinthoides seems largely dependent for its reproduction on a single native bird species. Although wind pollination is prominent in many of the trees in the Tasmanian rainforest (Read, 1999), and the subfamily Styphelioideae of the Ericaceae includes some species, such as *Richea procera* (F.Muell.) F.Muell. and *R. sprengelioides* (R.Br.) F.Muell. (Ladd, 2006), that use wind as a pollen vector, the large and colourful flowers of *P. cerinthoides* with their nectar and sticky pollen were not consistent with wind pollination. Although many of the understorey shrubs in the Tasmanian rainforest display an insect pollination syndrome (Read, 1999) few insects visited *P. cerinthoides*.

Although *P. cerinthoides* was self-compatible, the production of viable seed was markedly reduced by pollinator exclusion. In general autogamy is considered to provide some reproductive assurance for plants when potential mates or pollinators are scarce (Lloyd and Schoen, 1992). Artificial geitonogamy produced abundant viable seed so the stigma was

receptive in mature buds, as this was the stage used in the pollination trial. However, the separation of about 1 mm between anthers and stigma probably provided a barrier to autogamy in this species. Stigma exertion beyond the anthers is a pleisomorphic character in the family as it is considered to limit selfing in many other ericaceous species (Keighery, 1996). However, *Cosmelia rubra* R.Br. is bird-pollinated and physically able to self-pollinate (Keighery, 1996). In *Rhododendron*, where the stigma is also exerted beyond the anthers, few fruits were produced if pollinators were excluded but, as in *P. cerinthoides*, fruit set was high after artificial pollination (Kudo, 1993). Geitonogamy and autogamous selfing have the ecological properties of cross-fertilisation and genetic properties of self-fertilisation (Lloyd and Schoen, 1992). Cross-fertilisation is considered to be the preferred method for producing suitably fit progeny through avoidance of inbreeding depression (Jarne and Charlesworth, 1993) but many species tend to exhibit a mixed mating system (Goodwillie et al., 2005). This is the case with *P. cerinthoides* where the flower structure prevents, or at least discourages, autogamy but the size of flowers and flowering phenology means that geitonogamous pollination is equally or more likely than xenogamy.

In February, the flowering time of *P. cerinthoides* overlapped with the sympatric *Trochocarpa cunninghamii* and the eastern spinebill visited both species. After early March, *P. cerinthoides* was the only species in flower for the bird to visit, so pollen loss to other plant species was minimised. In the temperate rainforest environment, *P. cerinthoides* often occurs in dense patches. The pressure for an individual pollinator to continue foraging on a given plant may be expected to increase with a decrease in population density (Arroyo, 1976). Thus, an increase in xenogamy would be associated with greater population density and probably also with the peak in flowering. During peak flowering, the eastern spinebill was observed to move between several *P. cerinthoides* flowers on the same plant before moving between plants. It was not observed to visit all the flowers on any one plant. It is likely that this behaviour would change depending on the numbers of flowers available. Furthermore, as the level of geitonogamy versus xenogamy depends on the behavioural traits of a pollinator (Arroyo, 1976), it is likely that a bird-pollinated plant would have a lower level of geitonogamy than a plant pollinated by a less mobile animal. *Prionotes cerinthoides* was found to be pollen-limited (although the degree of limitation was not shown) confirming the suggestion of Lawrence (1992).

Flower visitors were of low frequency in the temperate rainforest compared with the bird-pollinated *Leptecophylla divaricata* which flowers in dry sclerophyll forest at the same time of year (Higham and McQuillan, 2000). *P. cerinthoides* was also subject to nectar-robbing by the crescent honeyeater, bumblebee and honeybee. In another of the Tasmanian long-tubed Ericaceae, *Epacris impressa* Labill., nectar-robbing by bumblebees was found to be more frequent in populations with longer corollas (Hingston and McQuillan, 1998). In contrast, bumblebees have been observed to access shorter-tubed Ericaceae species, such as *Epacris marginata* Melville (unpublished data KJ), in a manner that would ensure pollination. The length of the corolla tube may encourage nectar-robbing but the length of the bill and extended tongue of the eastern spinebill (27 mm, bill alone 22.5 mm) and the crescent honeyeater (25 - 32 mm, bill alone 16.1-18.0 mm) (Paton and Ford, 1977) are certainly long enough to access the nectar via the corolla mouth. Bill wiping on branches by the eastern spinebill was observed and this may decrease the effectiveness of pollen transfer (Cuthill et al., 1992). Competition for pollination services from other flowering plants seems unlikely, except perhaps for early in the season when *Trochocarpa cunninghamii* is flowering.

The nectar resource is likely to be more readily available to the eastern spinebill than to the crescent honeyeater because of a combination of pollinator behaviour and plant habit. *Prionotes cerinthoides* grows on slender, flexible branches and some flowers hang freely in the air. The stems are likely to bend and move considerably under the weight of a bird. The eastern spinebill accessed flowers either by hovering or from a perch. It manipulated flowers and manoeuvered its body to gain access via the corolla mouth. In contrast, the crescent honeyeater, like most honeyeaters, always used a perch (Pyke, 1980) and was less agile in its flower handling technique, usually nectar-robbing by creating holes or reusing existing holes at the base of the corolla away from the anthers and stigma. Hole reuse may explain why the majority of holes were larger later in the flowering season. Although the crescent honeyeater was not observed to behave as a pollinator during the course of this study, it cannot be ruled out of this role, as it is a known pollinator of another endemic Tasmanian epacrid, *Leptecophylla divaricata* (Higham and McQuillan, 2000). The crescent honeyeater has the potential to access nectar via the corolla mouth and to potentially pollinate *P. cerinthoides,* where flowers are accessible from a perch or ground (particularly in alpine and subalpine habitats where the plant scrambles over rocks). However, the requirement for a perch means that most honeyeaters (in Tasmania), other than the eastern spinebill (Pyke, 1980), would be

restricted in their access to the nectar resource via the corolla mouth and therefore restricted in their potential to be pollinators.

It is likely that *P. cerinthoides* is ecologically specialised (Fenster et al., 2004) to one functional group of native pollinators, currently including only the eastern spinebill. The introduced bumblebee may potentially pollinate *P. cerinthoides* and represents a different functional group. However, the bumblebee is a relatively recent introduction to Tasmania, since 1992 (Semmens, 1993), and has not evolved with *P. cerinthoides.* Ants occurred in flowers but are unlikely to be pollinators (Beattie, 1982). In *Prionotes,* ants could contact the anthers but contact with the stigma would be unlikely, as it is extended beyond the corolla mouth. As the stigma and anthers of *P. cerinthoides* are at the mouth of the corolla there is no specialisation that would prevent native or introduced invertebrates from affecting pollination. However, there are few pollinators that have a bill or proboscis length and agility to take nectar from the flowers via the corolla mouth.

It is possible that the flower of *P. cerinthoides* has evolved to enable pollination by the eastern spinebill. However, the absence of pollination information on an ancestral population or its immediate sister group makes it difficult to determine evolutionary specialisation. The phylogenetically-isolated clade comprising *Lebetanthus* and *Prionotes* are placed sister to the rest of the subfamily Styphelioideae (Crayn et al., 1998). Unfortunately, the pollination biology of *Lebetanthus myrsinites* (Lam.) Dusén is unknown (Mary Kalin-Arroyo, pers. comm. 2008), but its flowering time and floral morphology are different from *P. cerinthoides,* its closest living relative. *L. myrsinites* occurs in temperate rainforest in Chile and is summer-flowering rather than autumn-flowering. It has smaller white (eFloras, 2008) or pink to red flowers (flowering specimens obtained from the Allan Herbarium, Manaaki Whenua Landcare Research, Lincoln, New Zealand: CHR168375A, CHR168375B, CHR168376, CHR168381 included flowers that were crimson pink). *Lebetanthus myrsinites* has an average corolla length of about 5 mm (eFloras, 2008) much shorter than 20 to 25 mm of *P. cerinthoides* (Curtis, 1963). The floral morphology of *P. cerinthoides* aligns well with a bird pollination syndrome while *L. myrsinites* is potentially more consistent with an insect pollination syndrome (Faegri and van der Pijl, 1979). If the common ancestor of *P. cerinthoides* and *L. myrsinites* was insect-pollinated then it could be said that *P. cerinthoides* has undergone evolutionary specialisation, but this remains unproven.

In summary, it appears that the reproduction of *P. cerinthoides* depends largely on the services of a single native bird species, the eastern spinebill, and that this pollinator and limited selfing do not combine to result in a high degree of seed set. Although its floral structure makes it possible for other species to potentially act as pollinators, its habitat, habit and flowering time mean that there are few native animal species that can potentially provide this service. In Tasmania, both *P. cerinthoides* and the eastern spinebill are currently common and widespread. *Prionotes cerinthoides* is well-reserved in the Tasmanian Wilderness World Heritage Area and other National Parks where its habitat remains intact, while the eastern spinebill is widely distributed over the island, and attends other red-flowered epacrids, such as the relatively long-tubed *Epacris impressa* and *Astroloma humifusum* (Cav.) R.Br. The long term conservation of this association therefore seems secure.

2.6 Acknowledgements

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2.8 Appendix

Appendix 2-1 Insects on sticky traps (Note: N = traps facing towards and within 50 cm of *Prionotes,* A = traps facing away and 1 m distant. The Diptera (flies) were: Cera = Ceratopogonidae, Myce = Mycetophilidae, Scia = Sciaridae, Phor = Phoridae, Call = Calliphoridae, Doli = Dolichopodidae, Ceci = Cecidomyidae, Tipu = Tipulidae, Psyc = Psychodidae, Chir = Chironomidae; the Coleoptera (beetles) were: Stap = Staphylinidae – Aleocharinae, Scir = Scirtidae, Phal = Phalacridae, Mela = Melandryidae – *Orchesia* Latreille; the Hymenoptera (wasps) were: Proc = Proctotrupoidea, micro = microwasps, Brac = Braconidae; Psoc = Psocoptera (booklouse) ; Opil = Opiliones (daddylonglegs); Homo = Homoptera – Anilius (leaf hopper); the Collembola (springtail) was Paro = Paronellidae).

	Cera	Myce	Scia	Phor	Call	Doli	Ceci	Tipu	Psyc	Chir	Stap	Scir	Phal	Mela	Proc	micr	Brac	Psoc	Opil	Homo	Paro	Total
N1	0	0	0	3	0	1	0	0	0	0	3	0	0	1	0	0	0	0	0	0	0	8
N2	0	1	0	27	0	4	0	0	0	0	10	0	1	0	0	3	3	1	0	0	0	50
N3	0	0	0	13	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	14
N4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
N5	0	1	1	9	0	4	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	18
N6	1	4	0	20	0	0	4	1	0	0	7	0	0	0	0	2	0	0	0	0	0	39
N7	0	0	0	15	0	0	1	0	0	0	6	0	0	0	1	1	0	0	1	0	0	25
N8	0	0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	6
N9	1	1	0	14	1	2	1	0	0	17	5	0	0	0	0	2	0	0	1	0	0	45
N10	1	2	0	15	0	0	0	0	1	1	3	0	1	0	0	1	1	0	0	0	0	26
A1	0	0	0	6	0	0	0	0	0	0	3	0	0	0	0	1	0	0	0	0	0	10
A2	0	3	0	22	0	1	1	0	0	0	6	0	0	0	0	0	1	0	1	1	0	36
A3	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	4
A4	0	0	2	8	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	14
A5	1	0	0	5	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	9
A6	0	0	1	5	0	0	0	1	0	0	1	0	0	0	0	1	0	0	0	0	0	9
A7	0	0	1	6	0	1	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	11
A8	0	2	2	10	0	0	0	0	2	0	0	0	0	0	0	0	1	0	0	0	0	17
A9	1	0	0	11	0	2	0	0	1	9	3	0	0	0	0	2	0	0	0	0	0	29
A10	0	1	0	21	0	0	0	0	0	10	7	0	0	0	0	0	1	0	0	0	0	40

Chapter 3 Nocturnal mammals, diurnal lizards and the pollination ecology of the cryptic flowering *Acrotriche serrulata*

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3.1 Abstract

Acrotriche serrulata exhibits a complex and uncommon form of flowering. It starts with a male-phase flower that shows secondary pollen presentation on the perianth, and follows with a female-phase after the corolla is removed or abscissed. We examined the potential for insects, lizards and mammals to act as pollinators. Observations and experiments on breeding system, phenology, floral scent, flower visitors, and lizard feeding were undertaken in southern Australia. *Acrotriche serrulata* sets little fruit by autonomous-selfing but readily sets fruit after facilitated geitonogamy and xenogamy. Flower anthesis is diurnal and nocturnal. The nectar profile includes acetaldehyde, ethanol and ethyl acetate. The nocturnal mammals, *Trichosurus vulpecula* and the introduced *Rattus rattus*, were the only visitors observed to actively forage on the flowers. In contrast, the skinks *Egernia whitii*, *Niveoscincus ocellatus* and *N. metallicus*, routinely passed flowers full of nectar and only foraged on those presented during feeding observations. Insects visited the flowers but did not behave as pollinators. *Acrotriche serrulata* is likely pollinated by nocturnal mammals attracted to its flowers by scent. Effective pollinators appear to be rare over some of its range. This may have implications for the long-term reproductive success and conservation of *A. serrulata*.

3.2 Introduction

Floral diversity and complexity suggests coevolution and cospecialization of flowers and pollinators, however, there is evidence that complex flowers can have generalized pollination systems and receive multiple animal visitors (Waser and Ollerton 2006). The Australian endemic epacrid *Acrotriche serrulata* R.Br. undertakes secondary pollen presentation on the perianth (Howell et al. 1993). This is an uncommon occurrence, known from only a small number of plants including, *Trochetiopsis ebenus* Q.C.B.Cronk and *Dombeya cacuminum* Hochr. (Brodie et al. 2004; Prenner 2002). The presentation of pollen on organs other than the anthers occurs in about 20 dicotyledonous plant families (Ladd 1994). *Acrotriche serrulata* has two phases of flowering: a male-phase which includes secondary pollen presentation and a corolla full of nectar; and a female-phase consisting of stigma presentation after corolla removal or abscission (McConchie et al. 1986) (Fig. 3-1). *Acrotriche serrulata* is largely an outcrossing species with low levels of self-compatibility, suggesting that it requires a pollinator (Schneemilch and Steggles 2010). Insights into its breeding system are currently only available from the northern limits of its range on mainland Australia. There is no comparable information from the south, where it has been isolated on the island of Tasmania for over 10,000 years (Lambeck and Chappell 2001). There remains little information on the pollinators of this unusual plant.

Figure 3-1 from left: Male-phase *A. serrulata* flowers with secondary pollen presented (~ 5 mm wide); female-phase *A. serrulata* flowers (~ 3 mm wide)

Acrotriche serrulata sets flower over winter and early spring (Powell, 1992), a time of reduced insect activity throughout its range. While ants have been observed amongst the foliage of *A. serrulata*, none have been observed to carry pollen (McConchie et al. 1986) and they are seldom known to be effective pollen vectors (Beattie 1982; Peakall et al. 1990; Wagner 2000). It is possible that other insects may be attracted by the fragrance and nectar of *A. serrulata*. For instance, the beetle-pollinated (cantharophilous) syndrome includes a strong odour (Faegri and van der Pijl 1979). However, the corolla throat of *A. serrulata* is occluded with fine hairs that obstruct entry to the tube (McConchie et al. 1986), although it is possible that some ground-dwelling invertebrates, such as beetles, may have the strength to broach these hairs. *Acrotriche serrulata* is similar in its geoflorous habit to a number of other epacrids, including the co-occurring *Astroloma humifusum* (Cav.) R.Br.. However, in contrast to the cryptic clusters of *A. serrulata* flowers that are hidden amongst prickly leaves, *A. humifusum* has an ornithophilous pollination syndrome, indicated by the solitary, showy red corollas of its flowers (Curtis 1963; Faegri and van der Pijl 1979). *Acrotriche serrulata* does not provide obvious visual cues for bird pollinators but provides a nectar reward to potentially attract them.

Lizards and marsupials have previously been suggested as pollinators of *A. serrulata*. In captivity, *Niveoscincus metallicus* (O'Shaughnessy) (metallic skink) has been shown to consume *A. serrulata* flowers without damaging the female reproductive parts; and in the wild it has been found to carry pollen from a range of plant species, sometimes including a small amount (one to four pollen grains) of *A. serrulata* (Potter 2001). Although it seems likely that *N. metallicus* can play a minor role in pollination it has not been directly observed to forage on, or frequent, *A. serrulata* flowers in the wild (Potter 2001). However some lizards regularly forage on flowers elsewhere. In New Zealand, *Hoplodactylus* species (large geckos) eat nectar from flowers as diverse as the bottlebrush-like *Metrosideros excelsa* Gaertn., the large red flowers of *Phormium tenax* J.R.Forst. & G.Forst., the small, white flowers of *Myoporum laetum* G.Forst. and the white and lilac flowers of *Hebe bollonsii* Cockayne & Allan (Eifler 1995; Whitaker 1987). *Hoplodactylus* species can transport considerable amounts of pollen on their throats over many metres and for many hours providing opportunity to effect cross pollination (Whitaker 1987).

A small marsupial, *Antechinus stuartii* (Macleay) (brown antechinus), consumes the flowers of the related *Acrotriche aggregata* R.Br., and while seeking nectar it could effect pollination (Fletcher 1977; McConchie et al. 1986). Although it seems plausible that it could also play a role in the pollination of *A. serrulata*, *A. stuartii* does not occur in Tasmania, at the southernmost part of the range of the plant (Menkhorst and Knight 2001). Pollination by mammals that frequent the ground layer has been linked to flowers with some similarities to *A. serrulata*: geoflorous, dull-coloured and nectar-rich (Rourke and Wiens 1977; Wiens et al. 1983). Rodents have been shown to be effective pollen vectors for plants including some species of *Colchicum* (Kleizen et al. 2008), *Massonia depressa* Houtt. (Johnson et al., 2001), *Liparia parva* Vog. Ex Walp. (Letten and Midgley 2009), *Whiteheadia bifolia* Baker (Wester et al. 2009), and *Protea nana* Lam. (Biccard and Midgley 2009). Unlike *A. serrulata*, these flowers tend to be large and robust. In southern Australia, marsupials are also pollinators of some geoflorous Proteaceae, including some species of *Banksia, Dryandra, Isopogon* and *Petrophile* (Rourke and Wiens 1977).

Particular morphological features of flowers are associated with particular pollination systems (Faegri and van der Pijl 1979), although the correspondence is not always totally reliable (Hingston and McQuillan 2000). Given its cryptic habit and unusual flowering, it is likely that *A. serrulata* requires very specific pollinator behaviour to effect pollination (Howell et al. 1993; McConchie et al. 1986). The flowers of *A. serrulata* have similar attributes to some insect-pollinated, lizard-pollinated and mammal-pollinated plants, making it desirable to test for these three vectors.

We examined the potential for insects, lizards and mammals to act as pollinators of this plant in the southern-most parts of its range. We undertook observations and experiments on breeding system, phenology, floral scent, flower visitors, and lizard feeding.

3.3 Methods

3.3.1 Study sites

Experiments and observations were undertaken at two sites, each about one to 1.5 hectares in size within large areas of native vegetation, in southern Tasmania, Australia: *Eucalyptus*

pulchella dry sclerophyll forest at Chimney Pot Hill in Ridgeway Park (42º55'09"S 147º17'01"E) from Aug to Nov 2008 and Aug to Nov 2009; and Echo Sugarloaf State Reserve (43º14'43"S 147º07'52"E) from Aug to Dec 2009. Additional observations of potential pollinators were made in exposed cliff-top coastal vegetation at Cape Raoul in the Tasman National Park (43º13'05"S 147º47'05"E) in Sep 2008, and in *E. obliqua* dry sclerophyll forest at Randalls Bay Conservation Area (43º14'41"S 147º07'55"E) in Sep 2009. Vascular plant nomenclature follows Buchanan (2007).

3.3.2 Breeding system and open pollination experiments

These experiments were used to determine if *A. serrulata* is dependent on pollinators for fruit set and seed production. In 2008, an autonomous-selfing and open pollination (open control) experiment was run at Chimney Pot Hill. In 2009, autonomous-selfing, open pollination, hand-selfing (geitonogamy) and hand-crossing (xenogamy) experiments were undertaken at Chimney Pot Hill and autonomous-selfing and open pollination experiments at Echo Sugarloaf. *Acrotriche serrulata* plants with good bud set were selected and randomly assigned to a treatment. Buds were bagged in mid August of each year and fruit set was recorded from late October to late November. To determine if the male-phase flowers required physical removal to present the female-phase, the autonomous-selfing treatment was also scored for the presence of male-phase flowers that had wilted in situ.

3.3.2.1 Autonomous- selfing

In early Sep 2008 at Chimney Pot Hill, 431 buds on 8 plants were bagged in terylene ¼ mm mesh bags to exclude all animal visitors from the flowers. Before bagging, any insects were physically removed. On 28 August 2009, a total of 312 flowers on 8 plants were bagged at Chimney Pot Hill and a total of 311 buds on 5 plants were bagged at Echo Sugarloaf.

3.3.2.2 Open control

In early September 2008 at Chimney Pot Hill, 331 buds on 8 plants were marked with green twist ties and left open to all pollinators. Twist ties were located on the same branch as the flower but distant from it to minimise any possible influence on flower visitors. On 28 August and 1 September 2009, 261 buds on 8 plants were tagged at Chimney Pot Hill and 319 buds at Echo Sugarloaf respectively.

3.3.2.3 Hand-selfing

In late August 2009, 201 buds on 8 plants were bagged at Chimney Pot Hill. When flowers were open and plump with nectar, their corollas were removed with tweezers to expose the gynoecium. Geitonogamous pollination was undertaken by bringing a corolla with pollen presented, into contact with the exposed stigma. To ensure the success of pollen transfer, the presence of pollen grains on the stigma was checked with a hand lens. Following treatment, terylene mesh bags were placed back over the flowers to exclude other visitors. Pollination was carried out once for each flower on 2, 10 or 22 September 2009. As pollen dehisced before buds opened, no emasculation was done. The time of stigma receptivity was unknown; so we removed the corolla when it was plump with nectar (a time when an animal would be most likely to forage on it) and deposited pollen on the stigma at the same time.

3.3.2.4 Hand-crossing

206 buds from 8 plants were bagged and pollinated in the same fashion, and at the same times, as for the hand-selfing treatment, except that pollen was transported from a flower on an *A. serrulata* plant growing at least three metres away.

3.3.2.5 Open pollination

We collected fruit to examine seed set from open pollination. At Echo Sugarloaf we selected 10 plants with fruit present. We randomly collected 20 fruit per plant by tossing a 35 by 35 cm quadrat frame onto the plant and collecting from random positions within it. A preliminary survey at Chimney Pot Hill revealed that very few fruit were present. The 10 plants chosen were found to contain a total of 96 fruits compared with the same plant area sampled from 10 plants at Echo Sugarloaf which contained 262 fruit. We minimised our fruit removal from Chimney Pot Hill by collecting a total of 20 fruits from 15 plants. The first two fruits observed upon arrival at a plant were collected, unless only one was present.

3.3.3 Phenology

A total of 250 flowers were scored every two weeks from 9 September to 16 October 2008 at the Chimney Pot Hill site. A minimum of five plants were chosen, with at least three metres between them. A bud, male-phase flower, female-phase flower or fruit was randomly chosen on each plant and the 49 closest to it were then scored.

Flower anthesis was examined from 11 to 17 September 2009 at Chimney Pot Hill and 6 to 7 September 2009 at Echo Sugarloaf. Eleven plants and 247 buds and five plants and 319 buds (the control for the breeding systems trial) were used at the sites respectively. A flower was deemed open when the corolla lobes split apart revealing the secondary presenters.

3.3.4 Flower visitors

Over 430 hrs of observations were undertaken to determine what animals visit *A. serrulata* flowers. Male-phase flowers with nectar and pollen present were chosen for observation. Over 50 hrs of diurnal observations were made in person and by video camera (Panasonic Digital Video Camera, model no. NV-GS70, 1.7 MP, 500x digital zoom), on a tripod, on over 700 flowers (~14 per hour) during peak flowering at Chimney Pot Hill (9, 10, 17, 18 and 19 September, 2, 11, 12 and 16 October 2008; 2 and 10 September 2009), Echo Sugarloaf (1 September 2009), Cape Raoul (11 and 24 September 2008) and Randalls Bay Conservation Area (23 September 2008). All diurnal observations were for insects, lizards and mammals. In addition, continuous diurnal/nocturnal observations were undertaken, for mammals and birds with a motion triggered camera (Scouting / Trail Digital Video Camera, model no. DTC-530V, 5 MP, waterproof, IR-LED for night operation and quick response motion triggered PIR (<1.2 sec)) strapped to a tree and trained on a plant with a minimum of 100 flowers for 130 hrs at Chimney Pot Hill (15-21 September 2009) and 250 hrs at Echo Sugarloaf (28 September-7 October 2009). Nocturnal observations were also made for all animals, including insects, using a video camera with built in light (JVC Digital Video Camera, model no. GZ-MG465, 1.07 MP, 32x optical zoom) set up on a tripod. The camera was trained on a minimum of 30 flowers for 3 hrs at Randall's Bay (1 September 2009), about 50 flowers for 8 hrs at Echo Sugarloaf (5 September 2009) and about 30 flowers for 2.25 hrs at Chimney Pot Hill (2 September 2009). Spotlighting for all animals was done at the same time as the nocturnal videoing (except 5 September when 3 hrs only were undertaken). A pollinator was defined as an animal that collected pollen and deposited it onto conspecific stigmas of other plants (Pellmyr 2002). A flower visitor was an animal at a flower that either did not contact the reproductive parts and/or did not travel between plants.

We undertook a qualitative assessment of the ground-dwelling insect fauna for the presence of potential pollinators. Twelve pitfall traps were used to sample the ground-dwelling insect fauna during the peak flowering period at Chimney Pot Hill from 9-17 September 2008. Traps were made from plastic drinking cups (9 cm deep and 7 cm diameter at the mouth) ⅓ filled with a 50% ethylene glycol and 50% water mix. These were placed within 15 cm of plants.

3.3.5 Feeding observations

We used feeding observations to assess if skinks eat or take nectar from *A. serrulata* male-phase flowers and other contemporaneous flowerers, *Leucopogon collinus* (Labill.) R.Br., *Epacris impressa* Labill. and *Astroloma humifusum*, in their natural environment. The flowers were presented separately and randomly (hanging from transparent nylon fishing line at the end of a stick) to opportunistically encountered basking skinks. As skinks are largely insectivores, meat (bacon) was often presented after the flowers to assess if lizards were receptive to feeding during flower presentation. Skink reaction was recorded as no interest or interest, where interest was deemed to include immediate interest (as soon as flowers were placed near them) and delayed interest (within 10 sec of presentation). We recorded how interested skinks interacted with flowers: grabbing, holding, biting, licking and corolla removal; and if the flowers were damaged. Clusters of 5 to 20 *A. serrulata* flowers were presented 65 times: 13 times to *Egernia whitii* (Lacepède) (White's skink); 41 times to *Niveoscincus ocellatus* (Gray) (ocellated skink); and 11 times to *Niveoscincus metallicus* (metallic skink). Clusters of the small white, hairy flowers of *L. collinus* were presented 34 times: 8 times to *E. whitii*, 21 times to *N. ocellatus* and 5 times to *N. metallicus*. Two to six large, red tubular flowers of *E. impressa* or *A. humifusum* were presented 39 times: 9 times to *E. whitii*, 25 times to *N. ocellatus*; and 5 times to *N. metallicus*. Bacon was presented 31 times: 8 times to *E. whitii*, 20 times to *N. ocellatus* and 4 times to *N. metallicus*. Feeding experiments were carried out on four warm days when skinks were active at the Chimney Pot Hill site (2, 11, 12 and 16 Oct 2008). The temperature, wind speed and cloud cover were recorded at the start of each period of the feeding observations and later if conditions changed.

3.3.6 Floral scent and nectar availability

Floral volatiles were sampled by solid-phase microextraction (SPME) and analysed by combined gas chromatography-mass spectrometry (GC-MS). SPME sampling was undertaken by exposing ~10 flowers in a sealed 2mL autosampler vial to a 75micron Carboxen-PDMS SPME Fibre (Supelco) held in a manual SPME syringe for a period of 5 minutes at room temperature. Volatiles collected on the SPME fibre were directly desorbed in the injection port of a Varian 3800 GC fitted with a Varian 1177 split/splitless injector at a temperature of 280C for 5 minutes with a split ratio of 8:1. During desorption period the GC oven was held at 35C, and it was then ramped linearly to 250 at 10C/minute. The effluent from the GC was directly coupled to a Varian 1200 triple quadrupole mass spectrometer, with the transfer line held at 280C and the ion source at 220C. Electron ionisation at 70eV was employed, and mass spectra were recorded over the range *m/z* 35 to 400 3 times per second. Identification of the compounds present was based on both the NIST Mass Spectra Database, a range of in-house libraries of volatile compounds, and known retention orders.

An indication of nectar availability in the morning (9.00 – 9.30 am on 10 September 2008 - a cool and cloudy day at Chimney Pot Hill) was assessed using a microcapillary tube to access nectar from 50 flowers on 6 plants, selected in an *ad hoc* fashion. Flowers chosen were in the male-phase of flowering and had some pollen present on their secondary presenters. Variability in nectar due to time of flowering and weather were not assessed. Measurements represented the minimum nectar available at the time of sampling, as microcapillary tubes do not extract all (Kearns and Inouye 1993), and the constricted corolla required removal before the viscous nectar could be accessed, resulting in some nectar remaining on the flower.

3.3.7 Data analysis

We used the Chi-square test (χ^2) of significance to determine if there were differences in fruit set between breeding system and open pollination treatments. We used the Mann-Whitney Test with Bonferroni adjustment (0.05 (level of significance) / 8 (no. tests carried out) = 0.006 level of significance applied) to determine if there were differences in seed set per fruit between breeding system and open pollination treatments. Due to low fruit set, data from autonomous-selfing treatments at Chimney Pot Hill and Echo Sugarloaf were combined for seed set analyses; and data from the open control and open pollination were combined within

each site for seed set analyses. No emasculation was undertaken in the hand-selfing and hand-crossing pollination experiments. However, as autonomous-selfing was found to account for < 1% at Chimney Pot Hill, no correction was applied. All tests were performed in MINITAB 15.

3.4 Results

3.4.1 Breeding system and open pollination experiments

Breeding system treatment was found to be a significant factor in determining fruit set at Chimney Pot Hill (Table 3-1, Fig. 3-2). The two treatments with the highest fruit set, hand-selfing and hand-crossing, were significantly different from each other. There was no difference in fruit set between the autonomous-selfing treatments in 2008 and 2009 at Chimney Pot Hill but some difference between Chimney Pot Hill and Echo Sugarloaf. The autonomous-selfing treatment had the lowest fruit set (0.6% in 2009 and 0.5% in 2008 at Chimney Pot Hill), hand-crossing the highest (78.2%) and hand-selfing in between (33.8%). Fruit set from autonomous-selfing was higher (3.8%) at Echo Sugarloaf. Most fruits had five or six locules but rarely did all have seed. At Chimney Pot Hill on 24 October 2008 only 19.6% of the open control, in any stage of floral development, remained, and by 26 November 2008 fruit set was 0.3% (on 24 October 2008, the potential fruiting success - male and / or female-phase flowers showing signs of swelling at the base - was 3.6%). On 17 October 2009, 29.1% of the open control remained and on 19 November 2009 the fruit set was 0.4%. At Echo Sugarloaf on 2 December 2009 fruit set was 0.3%.

The profile of seed set from fruit varied between treatments (Table 3-2). In the autonomous-selfing treatment seed set per fruit was significantly lower than in the hand-selfing and hand-crossing treatments. Hand-crossing resulted in significantly more seed set per fruit than any other treatment. Hand-selfing and hand-crossing had significantly more seed set per fruit than open pollination. Seed set per fruit resulting from open pollination was not significantly different between the study sites. At Chimney Pot Hill (2009), autonomous-selfing resulted in one seed in each of the two fruits produced; hand-selfing had a seed set per fruit range of 0-5; and hand-crossing had a range of 0-6. At Echo Sugarloaf, autonomous-selfing had a seed set

range of 1-3 in the six fruit produced. At Echo Sugarloaf, open pollination had a range of 0-5; and at Chimney Pot Hill a range of 0-4. Male-phase flowers, bagged for autonomous-selfing treatments, generally wilted in situ (92.1% and 97.7% of flowers wilted at Chimney Pot Hill in 2008 and 2009 respectively, and 87.7% at Echo Sugarloaf in 2009).

Table 3-1 Significance of differences in fruit set between treatments (bold type indicates significant difference <0.05)

Year	Treatment 1 vs. 2	No. flowers in treatment 1 (% fruit set)	No. flowers in treatment 2 (% fruit set)	χ^2	DF	*P*
2009	Autonomous-selfing vs hand-selfing	311 (0.6%)	200 (33.2%)	113.936	1	**<0.001**
2009	Autonomous-selfing vs hand-crossing	311 (0.6%)	160 (78.2%)	463.210	1	**<0.001**
2009	Autonomous-selfing vs open control	311 (0.6%)	261 (0.4%)	0.181	1	0.670
2009	Hand-selfing vs hand-crossing	200 (33.8%)	160 (78.2%)	81.225	1	**<0.001**
2009	Open control vs hand-selfing	261 (0.4%)	200 (33.8%)	99.993	1	**<0.001**
2009	Open control vs hand-crossing	261 (0.4%)	160 (78.2%)	417.788	1	**<0.001**
2008 vs. 2009	Autonomous-selfing vs autonomous-selfing	432 (0.3%)	311 (0.6%)	0.107	1	0.743
2009	Autonomous-selfing vs autonomous-selfingES	311 (0.6%)	152 (3.8%)	6.594	1	**0.010**
2009	Autonomous-selfingES vs open controlES	152 (3.8%)	290 (0.3%)	8.304	1	**0.004**

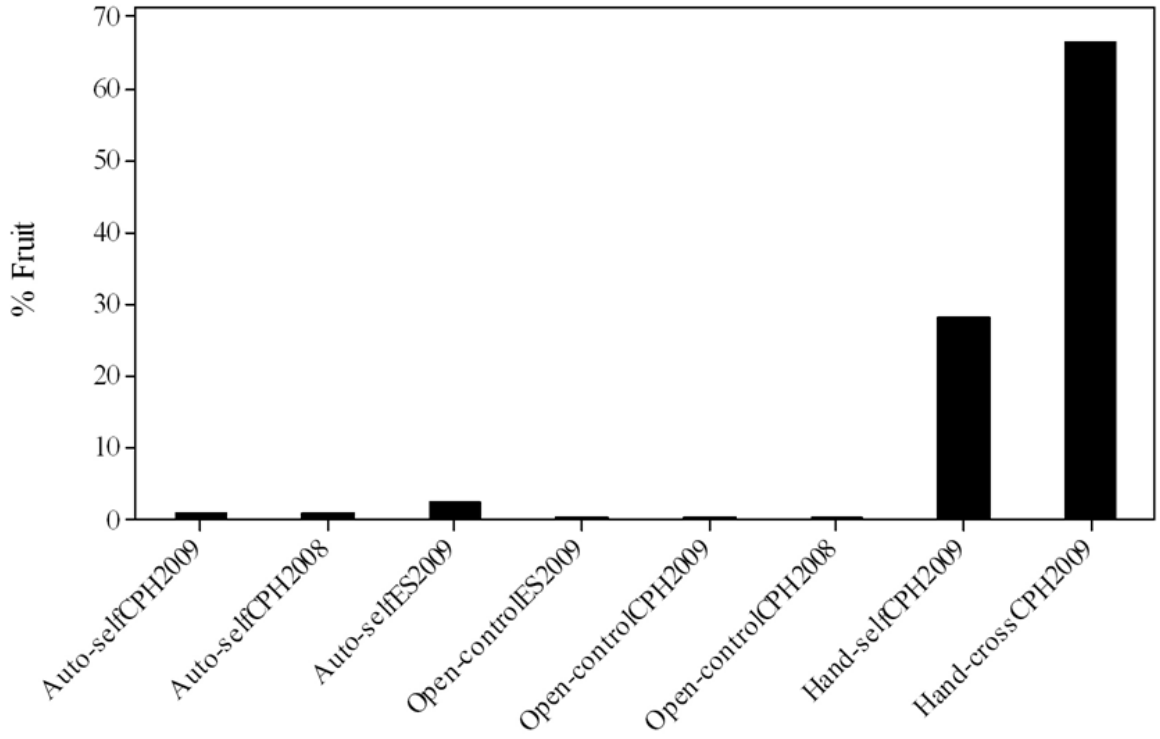

Figure 3-2 Fruit set for breeding system treatments and open-controls (CPH = Chimney Pot Hill and ES = Echo Sugarloaf)

Table 3-2 Significance of differences in seed set per fruit between treatments in 2009 (P – adjusted for ties; bold type indicates significant difference with Bonferroni adjustment <0.006)

Treatment 1 vs 2	No. fruit in treatment 1 (median seed set per fruit)	No. fruit in treatment 2 (median seed set per fruit)	W	*P*
Autonomous-selfing* vs hand-selfing	17 (1)	68 (2)	450.0	**0.0014**
Autonomous-selfing* vs hand-crossing	17 (1)	160 (4)	389.5	**<0.0001**
Autonomous-selfing* vs open pollination	17 (1)	22 (1)	409.0	0.0261
Hand-selfing vs. hand-crossing	68 (2)	160 (4)	20483.0	**<0.0001**
Open pollination vs hand-selfing	22 (1)	68 (2)	528.0	**<0.0001**
Open pollination vs hand-crossing	22 (1)	160 (4)	558.0	**<0.0001**
Open pollination vs open pollinationES	22 (1)	199 (1)	2187.5	0.3423
Autonomous-selfing* vs open pollinationES	17 (1)	199 (1)	1964.5	0.1656

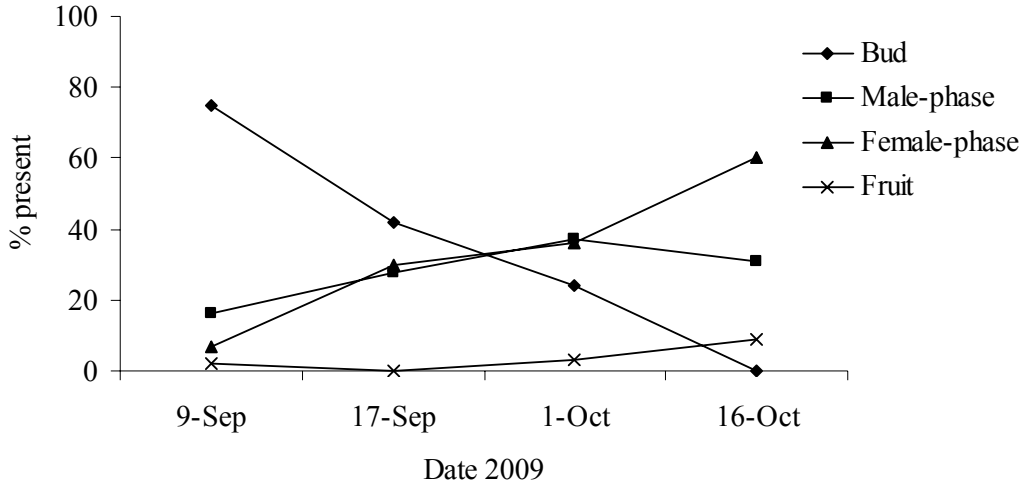

Figure 3-3 Phenology. (Note that by the 16 Oct sampling all male-phase flowers were wilted, no longer had nectar present and would fall from the plant upon touching. Also by this time, female-phase flowers were wilted and showed no sign of fruit developing)

3.4.2 Phenology

The first flowers were observed in the last week of August at Randalls Bay Conservation Area and at Chimney Pot Hill during the first week of September (Fig 3-3). Flowering effectively finished in the last week of October. Buds declined from 75% in early September to 24% in early October and were effectively absent by mid October. Male-phase flowers were at about 16% in early September, 28% in mid September, 37% in early October and almost absent by mid October (although 31% were recorded, all were wilted and none had nectar). Wilted male-phase flowers were first observed in mid September and would fall from the bush upon touching. Female-phase flowers were present in low numbers in early September (7%), increased to 30-36% in mid September and early October respectively and reached 60% in mid October. Fruit set was 2% in early September and increased to 9% in mid October. As the aborted, wilted male-phase flowers were unaccounted for, the proportion of female-phase flowers and fruit were likely to have been inflated. Male-phase flowers with nectar often remained in the field for days at a time. A branch of 27 flowers used for videoing was observed to remain plump with nectar for at least four days before the corollas were removed; and during day and night observations most flower clusters remained for greater than a week.

Flower anthesis occurred during the day and night. Of the 247 buds observed at Chimney Pot Hill over a one week period, 51 opened during the day and 21 overnight. Of the 319 buds observed at Echo Sugarloaf over 48 hours, 20 opened at night and none during the day. Flowers completely disappeared (probably removed by animals) during the day and night (16% night, 8% day at Chimney Pot Hill; and 7% night, 8% day at Echo Sugarloaf). Pollen presenters and corollas were subject to herbivory by invertebrates such as the caterpillar *Poecilasthena* species (Geometridae).

3.4.3 Flower visitors

Acrotriche serrulata received three night visits: one from *Trichosurus vulpecula* (Kerr) (common brushtail possum) at 20.32 pm on 17 September 2009 at Chimney Pot Hill; and two from the introduced *Rattus rattus* (L.) (black rat) at 20.00 pm on 24 September and 3.38 am on 25 September 2009 at Echo Sugarloaf. *Rattus rattus* was identified from the video footage as distinct from related native rodents by its tail length which exceeds its body length (Menkhorst and Knight, 2001). These were the only visitors observed, in the wild, to feed on *A. serrulata* flowers. Both *T. vulpecula* and *R. rattus* moved about *A. serrulata*, feeding in numerous places on the plant. Although *T. vulpecula* was not observed foraging at Echo Sugarloaf, its droppings were present on and near some *A. serrulata* plants.

Trichosurus vulpecula was observed to feed by poking its nose amongst the prickly leaves to locate the hidden flowers, opening the foliage with its hands, holding the foliage down and placing its face into the plant. It was not observed to use its hands to remove flowers for feeding. *Rattus rattus* moved over a large area of the plant on each occasion, constantly foraging by poking its nose in amongst the prickly leaves. The flowers are too small and hidden to be observed on our night video footage, so to assist in determining whether feeding by these animals was destructive (such as complete flower removal) or useful for pollination (corolla removal leaving intact female-phase of flower) we examined the plants in the areas visited (as observed on video footage). We found a few female-phase flowers exposed where *T. vulpecula* had visited but not enough to account for the amount of foraging observed. As very few flowers remained in the visited areas, including an absence of obvious flower-clusters present prior to the visit, it is highly likely that *T. vulpecula* removed the flowers. In contrast, many female-phase flowers were exposed in all areas visited by *R. rattus*.

No skinks were observed to forage on *A. serrulata* during the observations undertaken at times when they were actively foraging and moving about the environment. On over 50 occasions (including seven from video footage) they were observed to travel straight past or directly over clusters of *A. serrulata* flowers in full nectar and on numerous occasions they basked or hunted insects near plants without visiting the flowers. Two *N. metallicus* found dead in pitfall traps were dissected to see if their digestive systems contained *A. serrulata* material. The anterior gut of skink 1 (43 mm nose tip to cloaca and 56 mm tail) contained a whole *Lasioglossum* bee and a small beetle, and the posterior gut had a dolichoderine ant; the anterior gut of skink 2 (37 mm nose tip to cloaca and 53 mm tail) had a wolf spider Lycosidae - *Artoria* sp. and the posterior gut had a fly (Diptera) and part of a caterpillar, possibly Noctuidae. Thus, no *A. serrulata* material was found in their digestive tracts. Skinks were regularly observed to feed on insects. Flying insects present in the environment included known pollinators such as a wide array of native and introduced bees but these were not observed to visit *A. serrulata* flowers. Native flies (including those from the Phoridae, Sciaridae, Chironomidae, Mycetophilidae and Cecidomyidae), wasps (Braconidae), and ants (such as *Strumigenys* sp., *Prolasius*, *Pheidole* sp., *Myrmecia forficata* (Fabricius), several Dolichoderinae, and the night visitor, *Camponotus* sp.) were observed on plants. The rove beetle Staphylinidae-Aleocharinae and the moth Tortricidae are known pollinators of various other plant taxa, and occurred in pitfall traps (Appendix 3-1), but were not observed to visit *A. serrulata* flowers during our study. In general, ants would walk over a flower or feed on nectar leaking from either end of the corolla but were not observed to travel between flowers. Flies basked on or near flowers but usually did not contact the reproductive parts and were not observed to collect pollen. No birds were observed to visit the flowers in over 430 hrs of observation.

3.4.4 Feeding experiments

Feeding experiments were run in temperatures from 19 to 27 °C when skinks were foraging and hunting. It was found that skinks sometimes foraged on *A. serrulata* flowers that were presented to them (Figs 3-4 and 3-5). *Acrotriche serrulata* flowers were presented 65 times to skinks but on only four occasions (2 *E. whitii* and 2 *N. ocellatus*) were corollas removed exposing the female-phase of flowers. A total of 17 female-phase flowers were exposed and with one exception there was no damage to the styles or stigmas of flowers. An individual

E. whitii was responsible for removing 10 corollas and damaging one style and stigma. This individual was not interested when it was offered the *A. serrulata* flowers again five minutes later and still not interested after a further five minutes. Most flower and meat presentations were made to *E. whitii* and *N. ocellatus* as they were easier to approach than the smaller *N. metallicus*. The skinks interacted to varying degrees with the flowers and the bacon, with the bacon evoking the most interest and the most interactions, followed by *A. serrulata*. They were selective in their feeding but all species interacted with *A. serrulata* and the meat. *Niveoscincus metallicus* was not observed to interact with *L. collinus*, *E. impressa* or *A. humifusum*, although this may have been a product of a lower number of presentations. Overall, *L. collinus*, *E. impressa* and *A. humifusum* received less attention. However, one *N. ocellatus* became airborne in its effort to pull *L. collinus* down as it was being presented, licked the flower cluster a few times and then lost interest. Another *N. ocellatus* destructively consumed two whole *E. impressa* flowers. On one occasion, *N. ocellatus* showed no interest in the flowers presented to it, even when they were placed near it and then touching it. This individual subsequently caught a winged insect and consumed it.

3.4.5 Floral scent and nectar availability

The following major compounds were received for the floral volatiles: acetaldehyde; ethanol; ethyl acetate; 2,4,5-trimethyl dioxolane; ethyl 2-methylbutyrate; and ethyl hexa-2,4-dienoate (several isomers). Weaker signals were present for either vinyl acetate or butane-2,3-dione, methyl furan, 3-hydroxy 2 butanone, ethyl propanoate, ethyl isobutyrate, ethyl isopentanoate. Male-phase flowers were generally found to contain nectar in the morning (n = 50, range 0 - 5 µl, average 1.7 ± 1.2µl, median 1.5µl).

Figure 3-4 (Left to right): A-B. *N. ocellatus* forages on *A. serrulata* during feeding observations; C. *E. whitii* forages on presented *A. serrulata*; D. *E. whitii* with secondary pollen presenters contacting face during feeding observations

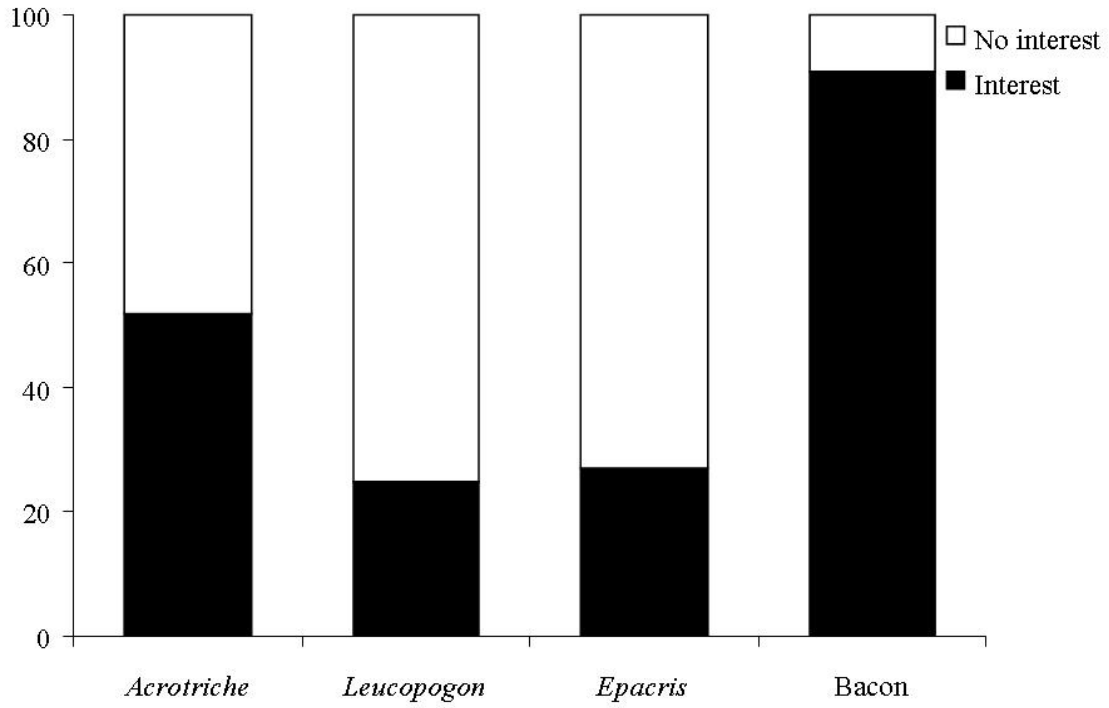

Figure 3-5 Percentage of interest of skinks in presented flowers and meat

3.5 Discussion

On the island of Tasmania, *A. serrulata* is largely dependent for its reproduction on an animal pollinator. This finding is consistent with research from the Australian mainland (Schneemilch and Steggles 2010). We found that although it is self-compatible it does not readily undertake autonomous-selfing – however, the amount appears to be variable between sites. It is likely that its two-phase approach to flowering provides a barrier to this type of selfing, but it does not appear to completely prevent it as previously suggested (Schneemilch and Steggles 2010). In our study, *A. serrulata* had between 0.6% (Chimney Pot Hill) and 3.8% (Echo Sugarloaf) fruit set from autonomous-selfing. We also found that when geitonogamy is facilitated, *A. serrulata* more readily sets fruit in the southern-part of its range (33.8% compared to the northern-part of its range 8.7% (Schneemilch and Steggles 2010)). However, our geitonogamy results fall within the range of between-plant variability noted by Schneemilch and Steggles (2010). In general, it has been shown that autonomous-selfing may confer reproductive assurance when opportunities for outcrossing are low (Jacquemyn and Brys 2008), and the association of isolated and island environments with self-compatible mating systems have been documented (Barrett and Shore 1987; Schueller 2004; Herlihy and Eckert 2005). Furthermore, it is suggested that the use of reproductive assurance mechanisms, such as autonomous-selfing, assists the maintenance of specialized pollination systems in pollinator-poor environments (Martén-Rodríguez and Fenster 2010). This, combined with its unusual flowering and complex pollinator requirements may mean that there is, at least some, reproductive backup from an ability to self-fertilize.

The two-phase flowering of *A. serrulata* plus the stigma submerged in nectar, and corolla hairs separating the stigma from the pollen, may reflect selective pressures to ensure high outcrossing rates. However, Fenster and Martén-Rodríguez (2007) found that delayed selfing was an important mechanism of reproductive assurance in species with either generalized or highly specialized pollination systems. In *A. serrulata*, it would be likely that any autonomous-selfing would have to occur when the stigma ceased to be submerged in nectar, thus it could be termed “delayed”. As Schneemilch and Steggles (2010) found that stigmas were no longer receptive in older flowers (defined in their study as those with faded-corollas) it is likely that the window of opportunity for autonomous-selfing to occur would potentially

be small (possibly reflecting the relatively low fruit set resulting from autonomous-selfing in our study).

The stickiness of the pollen (Schneemilch and Steggles 2010), and the location of the flowers low within the plant, precludes wind as a pollen vector. An animal is required to manipulate the flower in order to remove the corolla and present the female-phase of flowering. If not removed, the corolla usually wilts in situ. It is very likely that the animal that removes the corolla pollinates the flower, as there is no apparent reason for the rewardless female-phase to be visited again. However, an animal could affect pollination of a female-phase flower while foraging on an adjacent male-phase flower. Nocturnally active *Trichosurus vulpecula* and *R. rattus* were the only visitors observed to actively forage on the flowers of *A. serrulata*. In contrast, diurnal skinks routinely passed the flowers in full nectar without stopping to forage. The only skinks observed to forage on *A. serrulata* flowers were those manually presented with flowers during the feeding observations. They may have fed on presented flowers, dangling on the end of fishing line, as they appeared insect-like. This is in strong contrast to the behaviour of Tasmanian skinks on another Ericaceae species, *Richea scoparia* Hook.f., where on a warm day they readily and obviously remove the corolla and consume the nectar acting as potential pollinators or nectar robbers (Olsson et al. 2000; KJ unpublished data 2009). Similar to *R. scoparia*, *A. serrulata* has almost formed a calyptra as its corolla is effectively closed and requires removal. When skinks did forage on presented *A. serrulata* flowers they usually left the female reproductive structure intact, an important outcome of any potential interaction to affect pollination.

Trichosurus vulpecula and *R. rattus* are crepuscular and nocturnal and would most likely have been attracted to the *A. serrulata* flowers by smell. The flowers emit strong chemical signals including ethyl acetate and ethanol, both of which are fermentation products also emitted by baker's yeast (Goodrich et al. 2006). Elsewhere, other mammals are attracted by plant fragrances, including *Cynopterus sphinx* Vahl (short-nosed fruit bat) which is very attracted to ethyl acetate (Elangovan et al. 2006). Although the specific scents that attract particular animals are not known (Pettersson et al. 2004), it is possible that the scent profile of *A. serrulata*, incorporating ethyl acetate, is particularly attractive to mammals.

The amount of nectar associated with rodent-pollinated plants is reported to be variable, including *Massonia depressa* up to 182 µL; *Colchicum scabromarginatum* (Schltr. &

K.Krause) J.C.Manning & Vinn. about 58-210 µL; *C. coloratum* J.C.Manning & Vinn. about 17-24 µL; and *Liparia parva* about 10.7 µL (Johnson et al. 2001; Kleizen et al. 2008; Letten and Midgley 2009). The gecko-pollinated flowers of Pohutukawa (*Metrosideros excelsa*) have a volume of 26-62 µL (Eifler, 1995). The nectar volume in individual *A. serrulata* flowers is relatively low (about 5 µL) but the small flowers are clustered together enabling a larger volume of nectar to be quickly obtained by feeding on multiple flowers. *Acrotriche serrulata* flowers have between 10-50% sugar concentration (Potter 2001) and this is comparable to the variable sugar concentrations (ranging from 13-64%) associated with rodent-pollinated plants (Johnson et al. 2001; Kleizen et al. 2008; Letten and Midgley 2009) and also the gecko-pollinated Pohutukawa flowers with about 53% (Eifler 1995).

The secondary presentation of pollen on hairs located on the extremities of the petals appears to put pollen in a more prominent position for contact with an animal, while it locates flowers, than if the pollen remained on the anthers at the base of the petals. Unlike most flowers where the corolla is left intact by animal visitors and often visited multiple times, in *A. serrulata* the entire corolla (including pollen) is removed. Thus any chance of pollen dispersal relies on pollen adhering to an animal before the corolla is eaten or discarded. While foraging, *T. vulpecula* and *R. rattus* would come into contact with the secondary presented pollen on the perianth. Mammal fur provides an excellent surface for the transport of pollen (Rourke and Wiens, 1977). However, it is likely that *T. vulpecula* is a destructive feeder, and we cannot exclude the possibility that *R. rattus* may be also (Ecroyd et al. 1995; Pandit and Choudhury 2001). The disappearance of flowers from the open control and the flower anthesis experiment indicated that herbivory was occurring at both sites. Yet female-phase flowers were present in the areas where these mammals, particularly *R. rattus*, had been foraging. We also cannot rule out invertebrate visitors that were too small to trigger the motion sensor on our camera, although we did not observe them actively foraging on flowers during our other observations. The introduced *R. rattus* has not evolved with *A. serrulata* but is a known pollinator elsewhere. For example, *R. rattus* acts as an effective pollinator and occasional flower destroyer of the potentially bat-pollinated New Zealand root parasite, *Dactylanthus taylorii* Hook.f. (Ecroyd et al. 1995). In contrast, *T. vulpecula* is not known to be a pollinator and, through its browsing of inflorescences, threatens the survival of *D. taylorii* at most sites (Ecroyd et al. 1995). *Rattus rattus* is also a night visitor and pollinator of *Helicia nilagirica* Bedd. in southern India (Devy and Davidar 2003). In Australia,

Rattus fuscipes (Waterhouse) is one of the most important native pollinators of *Banksia spinulosa* Sm. (Goldingay et al. 1987). Like a brush flower, *A. serrulata* has externally presented pollen. An animal foraging on a brush flower tends to get pollen on its head and many parts of its body while clambering over the flower - *R. rattus* and *T. vulpecula* would be likely to get pollen on their faces while feeding on *A. serrulata*. They could also contact the pollen with their feet and bodies as they clamber over the plants, searching for flowers.

Over much of the flowering time of *A. serrulata* it was the main nectar resource present in its local environment. The phenology data shows that during early spring *A. serrulata* provides a nectar reward in over one-third of its flowers. However, these flowers could remain untouched and in full nectar, for over a week or until they withered, suggesting that the nectar was not highly prized at these locations. The breeding system results showed little or no significant difference in fruit set between autonomous-selfing and open pollination. Although it is likely that fruit may be removed from the open pollination plants as it becomes available, our regular checking of these plants tended to suggest that the actual fruit set was low. However, we found more fruit available on plants at Echo Sugarloaf than at Chimney Pot Hill when we randomly collected fruit from ten plants at each site. The apparent lack of interest in this large nectar resource and low fruit set, particularly at Chimney Pot Hill, may indicate the rarity or absence of a forager and pollinator. On the Australian mainland, *Antechinus stuartii* is known to consume and potentially pollinate *Acrotriche aggregata* (Fletcher 1977; McConchie et al. 1986). *Antechinus stuartii* does not occur in Tasmania. The Tasmanian congenors, *A. swainsonii* (Waterhouse) (dusky antechinus) and *A. minimus* (Finlayson) (swamp antechinus), both prefer areas of denser and wetter vegetation than is usually associated with *A. serrulata* (Menkhorst and Knight 2001). Specific surveys and trapping would be required to determine the presence or absence of *Antechinus* species. Other small mammals that potentially occur in the Tasmanian range of *A. serrulata* include *Rattus lutreolus* (Gray) (native bush rat), *Cercartetus lepidus* (Thomas) (little pygmy possum), *C. nanus* (Desmarest) (eastern pygmy possum), *Perameles gunnii* (Gray) (eastern barred bandicoot), *Isoodon obesulus* (Shaw) (southern brown bandicoot), *Petaurus breviceps* Waterhouse (introduced sugar glider) and *Mus musculus* L. (introduced house mouse).

Our evidence strongly suggests, albeit circumstantially, that *A. serrulata* is pollinated by a vertebrate, most likely a nocturnal mammal attracted by the scent of the flowers. Mammals are capable of removing the corolla – an important step in the pollination process. To date, we

know that *A. stuartii* (Fletcher 1977; McConchie et al. 1986), *T. vulpecula*, and *R. rattus* are attracted to the plant. The nectar contains ethyl acetate which attracts some mammals. It is possible that any pollination occurring maybe a by-product of destructive feeding. Throughout its wide range and across its interactions with many plant species, *T. vulpecula* is currently not known as a pollinator. The greater quantity of fruit set at Echo Sugarloaf (see methods section - open pollination) could either be a result of a different proportion (and possibly higher proportion) of *R. rattus* to *T. vulpecula* visiting flowers compared with Chimney Pot Hill; more prolific flowering; and / or the presence of another pollinator that was not observed. It is unlikely that skinks are directly involved in the pollination of *A. serrulata*, given their lack of interest in visiting flowers, although it is possible that their movement about plants may facilitate some pollen transfer. Overall, the observation of nectar-filled flowers remaining in the field for extended periods, and a low fruit set (particularly at Chimney Pot Hill), suggests that effective pollinators are rare over some of the range of *A. serrulata*. It is possible that the main native pollinator is absent from these sites. It is possible that the introduced *R. rattus* has moved into this niche. If this is the case, then there are implications for the long-term reproduction and conservation of *A. serrulata*. Fenster and Martén-Rodríguez (2007) suggest that over some part of a plant's geographic range and evolutionary history it is likely to experience pollinator limitation. They postulate that this will favour evolutionary shifts in pollination systems from specialized to generalized or *vice versa* and may result in selection of floral traits to attract alternative or additional pollinators. Having established mammals as potential pollinators, our next step is to determine if native pollinators are present elsewhere in the plant's range, or whether their rarity or absence is widespread.

3.6 Acknowledgements

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3.8 Appendix

Appendix 3-1 Animals in pitfall traps

The Hymenoptera (ants) were: *Strumigenys*, *Pheidole*, *Myrmecia forficata*, Dolichoderinae, *Prolasius*, Braconidae, Mycetophilidae; the Diptera (flies): Phoridae, Sciaridae, Chironomidae, Cecidomyidae; a grasshopper: *Tasmaniacris*; the Coleoptera (beetles) were: Alticinae, Pselaphidae, Staphylinidae – Aleocharinae, *Eulagria grandis* (larva), *Agrypnus*, *Onthophagus fuliginosus*, Promecoderus carabid; weevils: Amycterinae, Mandalotus; the mite was: Acarina; the Arachnida (spiders) were: Salticidae, Lycosidae – *Artoria sp.*, Lycosidae – *Venatrix sp.*, Amaurobiidae, Micropholcomatidae, Linyphiidae, Gnaphosidae; the *Collembola* (springtails) were: Hypogastrura; the millipedes were: black millipede, brown millipede; the *Lepidoptera* were: Geometridae (larva), the earwig: Dermaptera, Bug: Rhyparochrominae; Flea: Siphonoptera; Mollusca: Slug; Scorpion: *Cercophonicus squamea*; Moth: Tortricidae; the Hemiptera: Cicadellidae, Reduviidae; Cockroach: Blatellidae.

Chapter 4 Comparative floral presentation and bee-pollination of two *Sprengelia* spp.

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4.1 Abstract

Pollination by sonication is unusual in the Styphelioideae, family Ericaceae. *Sprengelia incarnata* and *S. propinqua* have floral characteristics that suggested they might be adapted to buzz pollination. Both species have florally similar nectarless flowers except that the stamens of *S. propinqua* spread widely after the flower opens, while those of *S. incarnata* cohere in the centre of the flower. To test whether sonication occurs, we observed bee behaviour at the flowers of both plant species, documented potential pollinators, and examined their floral and pollen attributes. We found that *S. incarnata* had smaller and drier pollen than *S. propinqua*. We found that *S. incarnata* was sonicated by native bees in the families Apidae (*Exoneura*), Halictidae (*Lasioglossum*) and Colletidae (Leioproctus, Euryglossa). While *S. propinqua* was also visited by bees from the Apidae (*Exoneura*) and Halictidae (*Lasioglossum*) pollen was collected by scraping. The introduced *Apis mellifera* (Apidae) foraged at *S. propinqua* but ignored *S. incarnata*. The two *Sprengelia* species shared some genera of potential pollinators, but appeared to have diverged enough in their floral and pollen characters to elicit different behaviours from the native and introduced bees.

4.2 Introduction

The interactions between plants and pollinators are thought to be responsible for much of the diversity in angiosperm flower morphology, with many floral traits associated with particular animal behaviours (Lawrence et al., 2001). The flowers of buzz-pollinated plants are a notable example of this phenomenon. Furthermore, it has been proposed that flowers with small, dry pollen typical of buzz-pollination may represent a transitional stage to anemophily (Buchmann, 1983), and phylogenetic analyses suggest that the evolution of anemophily is more likely in groups with these traits (Culley et al., 2002).

Buzz-pollination is widespread among angiosperms. In the Australian flora, buzz pollination is known to occur in a range of genera including *Hibbertia* (Bernhardt, 1984; Bernhardt, 1986), *Thelymitra* (Bernhardt and Burns-Balogh, 1986), *Dianella* (Bernhardt, 1995), *Tetratheca* (Driscoll, 2003) and *Solanum* (Anderson and Symon, 1988). In the Styphelioideae, buzz-pollination has been confirmed for *Conostephium* and hypothesised for *Coleanthera myrtoides* Stschegl., *Rupicola* species, some *Leucopogon* species, *Richea milliganii* (Hook.f.) F.Muell., and *Sprengelia incarnata* Sm. (Houston and Ladd, 2002; Ladd, 2006). A range of floral characteristics that make up the traits of buzz-pollinated flowers have been elucidated (Buchmann, 1983; Harder, 1990). In the Australian taxa, buzz-pollinated flowers have been observed to have either exposed anthers (solanoid-type), or anthers hidden by the petals (Houston and Ladd, 2002). They typically have purple or blue petals and yellow anthers, or white petals and purple anthers (Houston and Ladd, 2002).

Buzz pollination occurs when a bee vibrates its thoracic flight muscles over the anthers, vibrating dry pollen onto its body (Harder, 1998; Houston and Ladd, 2002; Thorp, 2000). Buzz pollination or sonication of flowers by bees has been associated with porocidal anthers such as those in the Ericaceae. Most Ericaceae have two-lobed anthers that dehisce by introrse or terminal pores (Curtis, 1963; Stephens, 2004), an important preadaptation to buzz pollination in ericads such as *Vaccinium stamineum* L. (Cane et al., 1985). In contrast to the rest of the Ericaceae, the subfamily Styphelioideae (epacrids) generally have unilocular anthers that open by a single longitudinal slit (Curtis, 1963). However, the more basal genera, *Prionotes*, *Sprengelia* and *Richea* can have bilocular anthers that dehisce by a single slit (Crayn et al., 1998; Curtis, 1963) giving them characteristics of both ericads and epacrids.

Although the epacrids do not have apically porose anthers, the following characters are thought to make the widespread *S. incarnata* a candidate for sonication: nectarless flowers, anthers that dehisce introrsely from an elongated pore, and stamens that cohere and move as a unit (Houston and Ladd, 2002). With the exception of this last character, the Tasmanian endemic *S. propinqua* A.Cunn. ex DC. shares these attributes. Until recently, *S. incarnata* and *S. propinqua* were considered to be a single variable species (Buchanan, 2009; Buchanan, 2005; Curtis, 1963). However, the stamens in *S. propinqua* are free (Curtis, 1963), generally separating and spreading away from the central position after the flower opens.

Sonication has been observed to occur, regardless of different stamen morphologies and arrangements. For instance, in Java, *Xylocopa* bees buzz-pollinate three *Dillenia* species: *D. suffruticosa* Martelli, where the stamens form a cone; and *D. alata* (D.C) Martelli and *D. philippinensis* Rolfe which have spreading stylar branches and both long and short stamens. On this evidence, *S. propinqua* may also be a candidate for sonication (Endress, 1997). We tested the hypothesis that the flowers of *S. incarnata* and *S. propinqua* are sonicated by native bees. We examined floral morphology, pollen tackiness, and documented the potential pollinators.

4.3 Methods

4.3.1 Study Species and Sites

Sprengelia propinqua was split from *S. incarnata* based on floral characters including free rather than cohering stamens and solely white flowers rather than bi-coloured pink and white flowers (Curtis, 1963; Walsh and Entwisle, 1996). The flowers of both species are hermaphroditic and nectarless. *Sprengelia propinqua* is a prominent species in moorland in southwest Tasmania while *S. incarnata* is a locally dominant species occurring throughout southeastern Australia. Observations on *S. incarnata* were made in buttongrass (*Gymnoschoenus sphaerocephalus* (R.Br.) Hook.f.) hummock sedgeland in the Peter Murrell Nature Reserve (43°00'45"S 147°18'43"E); in heathy *E. tenuiramis* Miq. woodland with buttongrass present in the understorey near Egg and Bacon Bay (43°14'45"S 147°06'19"E); in similar vegetation on the Tasman Peninsula (43°01'23"S 147°53'41"E) and in the Tasmanian

Wilderness World Heritage Area (WHA) where it co-occurs with *S. propinqua* (42°57'18"S 146°21'23"E). Observations on *S. propinqua* were made in buttongrass hummock sedgeland in the WHA (42°55'26"S 146°21'34"E and 42°53'03"S 146°22'52"E). An *Apis mellifera* L. (honeybee) hive was present within 100 m of one *S. propinqua* study site.

For the purpose of our study plants closely fitting the descriptions of *S. incarnata* and *S. propinqua* were chosen for examination (Curtis, 1963; Walsh and Entwisle, 1996). Plants with intermediate floral morphology occur in the western study area. Vascular plant nomenclature follows Buchanan (2009); and author names follow those on The International Plant Names Index (www.ipni.org – accessed 19 May 2010). Monthly climate averages for rainfall, temperature, relative humidity and wind speed for our study sites are given in Table 4-1.

Table 4-1 Monthly climate averages for *Sprengelia* sites (Note: All figures are from the closest climate stations on the Bureau of Meteorology website (www.bom.gov.au/weather/tas - accessed on 18 May 2010. These were Hobart, Dover, Port Arthur, and an average from the Strathgordon and Maydena Post Office climate sites).

Species and sites	Flowering time	Rainfall mm	Days rain ≥ 1 mm	Wind km/h 3 pm	Temp °C 3pm	% RH 3 pm
Sprenglia incarnata (Peter Murrell)	Sept - Oct	61	9	18	15	56
Sprenglia incarnata (Egg and Bacon Bay)	Sept - Oct	85	7	15	14	63
Sprenglia incarnata (Tasman Peninsula)	Sept - Oct	104	14	22	12	65
Sprenglia incarnata (WHA)	Sept - Oct	161	16	11	13	62
Sprengelia propinqua (WHA)	Oct – Nov	161	16	11	13	62

4.3.2 Floral Morphology and Pollen

Twenty specimens of each species were randomly selected from material housed at the Tasmanian Herbarium, Hobart (Appendix 4-1). Floral morphology was compared by measuring (to an accuracy of 0.5 mm) sepal, petal, style, stamen and anther length under a dissecting microscope. We used the Student's 2-sample t-test to determine if there were significant differences in the size of floral parts of *S. incarnata* and *S. propinqua*. All tests were performed in MINITAB 15.

To determine if there were any differences in the pollen of *S. incarnata* and *S. propinqua*, pollen samples from a live plant of each species (from Peter Murrell Reserve and WHA sites respectively) were examined under a Scanning Electron Microscope (SEM) at 5000x magnification at the Central Science Laboratory at the University of Tasmania. Maximum pollen grain diameter and tackiness were recorded. Tackiness in *Sprengelia* species was determined by whether pollen grains occurred separately (dry) or adhered to each other (sticky).

4.3.3 Flower Visitors

Observations on flower visitors were made in person (while walking amongst flowers) and by video camera (Panasonic Digital Video Camera, model number NV-GS70, 1.7 mega pixel, 500x digital zoom) mounted on a tripod. A pollinator is defined as an animal that collects pollen and deposits it onto conspecific stigmas of other plants (Pellmyr, 2002). In contrast, a flower visitor is an animal at a flower that either does not contact the reproductive parts of the flower and/or does not travel between plants. For the purpose of our study, we defined a potential pollinator as an animal that we observed to contact the reproductive organs of a plant, actively removed pollen from the anthers, and move between conspecific species. In addition to the 'solanoid' *Sprengelia* flower-form that enables easy observation of insects contacting and removing pollen from the anthers, the *Sprengelia* pollen is different in colour from many of the sympatric co-flowering plant species including *Pimelea*, *Hibbertia*, *Aotus* and *Pultenaea*. Potential buzz pollination was identified by a bee hunching over a flower's anthers with wings held back along the line of its body, and by an audible buzzing (Harder, 1998; Houston and Ladd, 2002; Thorp, 2000).

Observations were made on clear and relatively warm (> 18 º C) days. Observations were made on *S. incarnata* between 10 am and 4 pm on 9 Oct 2008, 29 Sep, 4 Oct, 10 Oct and 8 Nov 2009 during its peak flowering period. Observations were made on *S. propinqua* between 11 am and 3 pm on 23 Oct, 1 Nov, 5 Nov, 13 Nov, 19 Nov 2008 and 8 Nov 2009 during its peak flowering period.

Samples of the foraging insects were collected by netting, or captured straight into a plastic screw-top container wetted with ethanol. Insects were killed and stored in screw-top vials with 70% ethanol. Bees were identified to genus under a dissecting microscope using the key

of Michener (1965) and the Hingston bee collection which holds specimens determined by Dr. Ken Walker (National Museum of Victoria). Together with potential pollinators collected during our survey, the Hingston collection is housed at the School of Geography and Environmental Studies Laboratory, UTAS. Flies were identified using Colless and McAlpine (1991) and butterflies using Braby (2004). Other invertebrates were identified using Zborowski and Storey (2003) and Daley (2007).

4.4 Results

4.4.1 Floral Morphology and Pollen

There was no overlap in the size of floral parts with *S. incarnata* being smaller than *S. propinqua* in all parts (Table 4-2). Observation of pollen under the SEM revealed that the grains of *S. incarnata* occurred separately, indicating that they were dry, while the grains of *S. propinqua*, commonly adhered to form clumps indicating that the pollen was sticky. *Sprengelia propinqua* pollen was larger (ca 10%) than *S. incarnata* pollen, but had a similar morphology (Fig. 4-1).

Table 4-2 Floral presentation in *S. incarnata* versus *S. propinqua* in Tasmania

Floral presentation	***S. incarnata***	***S. propinqua***
Floral measurements (mean mm ± SE)*		
- Stamen	3.0 ± 0.04	4.8 ± 0.16
- Anther	1.6 ± 0.06	3.4 ± 0.15
- Style	3.7 ± 0.06	6.0 ± 0.90
- Petal	4.2 ± 0.09	6.6 ± 0.25
- Sepal	4.5 ± 0.09	7.2 ± 0.22
Flower colour	bicoloured pink and white	white
Flower position on stem	terminal	terminal
Flower heads	upright	upright
Stamen position	cohering in centre of flower	spreading widely as flower matures
Flowering time	Sep-Oct-Nov	Oct-Nov

**S. incarnata* and *S. propinqua* are significantly different in the size of all floral characters as follows: stamen ($t = -10.98$, $P < 0.001$, $DF = 22$), anther ($t = -11.42$, $P < 0.001$, $DF = 24$), style ($t = -11.30$, $P < 0.001$, $DF = 22$), petal ($t = -9.35$, $P < 0.001$, $DF = 24$), sepal ($t = -11.04$, $P < 0.001$, $DF = 25$).

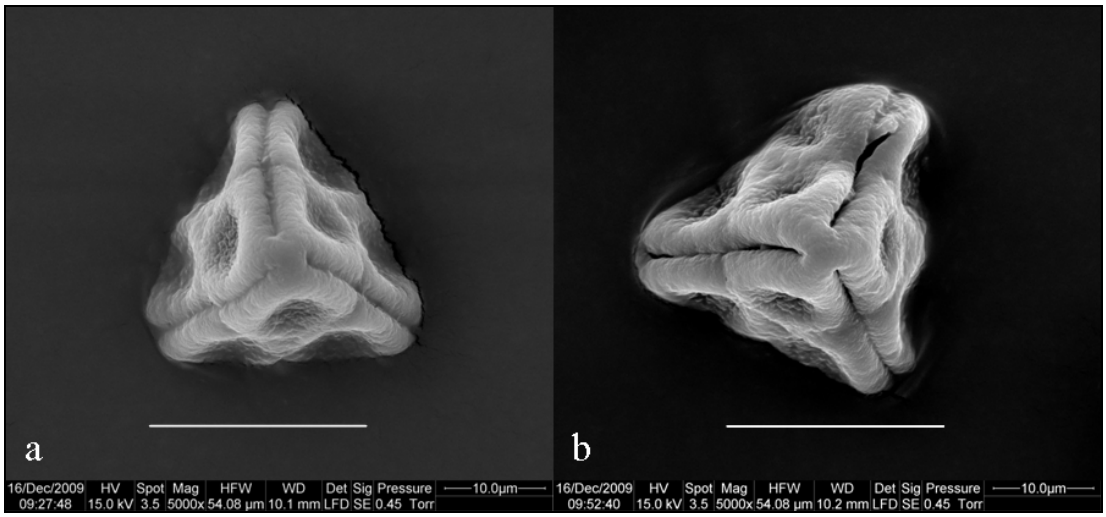

Figure 4-1 *Sprengelia incarnata* pollen grain; B. *S. propinqua* pollen grain (scale bar ~20 µm)

4.4.2 Flower Visitors

Sprengelia incarnata was repeatedly observed being sonicated by native bees (Fig. 4-2; Table 4-3). *Lasioglossum* species and *Exoneura* species were the main visitors (> 100 observations). During sonication the bees collected large amounts of pale-coloured pollen on their legs and bodies and moved between *S. incarnata* plants. A thick layer of pollen was collected on the hind legs (femur, tibia and basitarsus) and abdominal sternites with additional pollen also scattered on body hairs outside these areas, including the head. Bees were observed to groom themselves after sonication, moving pollen from the thorax to abdomen with the aid of the front legs. The pollen-covered abdomen was observed to contact a flower's stigma in a manner consistent with a potential pollinator (Fig. 4-2C). Hoverflies (Syrphidae) were present at the study sites and two individuals contacted the anthers and appeared to collect pollen from *S. incarnata*. However, they were not observed to move between *S. incarnata* flowers. An introduced bumble bee queen, *Bombus terrestris* (L.), was observed to visit five flowers but was not observed to collect pollen. *Apis mellifera* (honeybees) were present and active at all sites during observations. They visited three flowers of *S. incarnata* over three separate occasions but did not collect pollen. Generally they flew past *S. incarnata* without landing on the flowers. They frequently collected pollen from sympatric plants including *Euryomyrtus ramosissima* (A.Cunn.) Trudgen, *Leucopogon collinus* (Labill.) R.Br., *Pimelea linifolia* Sm., *Pultenaea stricta* Sims, and *Aotus ericoides* (Vent.) G.Don when native bees were buzzing *S. incarnata*. Native bees also visited some of these sympatric plants.

Figure 4-2 Lasioglossum (Parasphecodes) bees on *S. incarnata* showing: A. folded wing position; B. hunched position during sonication; C. pollen accumulation; and D. anther position in *S. propinqua*

No bees were observed to sonicate *S. propinqua*. *Apis mellifera* and *Lasioglossum* species were the main visitors (> 100 observations). Hoverflies (*Simosyrphus* species and *Melangyna* species) were also prominent visitors. *Exoneura* species was present but was only observed visiting *Epacris corymbiflora* Hook.f. Macleays' swallowtail butterfly (*Graphium macleayanus* (Leach)) visited *S. propinqua* and bobbed its head up and down in the same way it did to extract nectar from, and potentially pollinate, *E. corymbiflora*. On one occasion it probed a number of flowers on one plant. However, it was not observed to move between *S. propinqua* plants, making it a visitor, rather than a potential pollinator. In contrast, *G. macleayanus* regularly moved between *E. corymbiflora* plants (> 50 observations).

Graphium macleayanus is known to be predominantly a nectar feeder, which makes it unlikely to be a regular forager on, and pollinator of, the nectarless *S. propinqua*. During observations on 23 Oct and 1 Nov 2008 at the site near the apiary, *A. mellifera* was the only species active on *S. propinqua* and native bees were not observed.

Table 4-3 Potential pollinators and flower visitors of *Sprengelia*

(i) = introduced, p = potential pollinator, + = buzz pollination, fv = flower visitor

Animal	*S. incarnata*	*S. propinqua*
Bees		
Euryglossa sp.	p +	
Exoneura sp.	p +	
Lasioglossum (Chilalictus) sp.	p +	p
Lasioglossum (Parasphecodes) sp.	p +	p
Leioproctus sp.	p +	
(i) *Apis mellifera* L.	fv	p
(i) *Bombus terrestris* (L.)	fv	p?
Flies		
Syrphidae	p?	p
Musca vetustissima Walker		fv
Tachinid fly (long-legged)		fv
Tachinid fly (short-legged)	fv	fv
Butterflies and moths		
Junonia villida (Fabricius)	fv	
Graphium macleayanus (Leach)		fv
Melitulias graphicata (Walker)		fv
Beetles		
Elateridae	fv	fv
Paropsis sp.	fv	
Chauliognathus tricolor (Castelnau)		fv
Other		
Diaea sp.	fv	fv
Pentatomidae (unidentified shield bug)	fv	fv
Thripidae (unidentified thrip)	fv	fv
Curculionidae (unidentified weevil)	fv	

4.5 Discussion

We have confirmed that pollen is collected from *S. incarnata* by sonication, as predicted by Houston & Ladd (2002) and scraped from *S. propinqua*. *Sprengelia propinqua* was not observed to be sonicated. Regardless of differences in flower size, pollen tackiness, stamen morphology and arrangement, *S. incarnata* and *S. propinqua* have overlapping floral visitor profiles with *Lasioglossum* bees being prominent potential pollinators of both plants.

Sonication of *S. incarnata* is undertaken by at least four native bee genera in Tasmania. With the exception of *Euryglossa*, bees from these genera are known to sonicate a range of plants in Australia. *Leioproctus* species have been observed to buzz *Conostephium drummondii* (Stschegl.) C.A.Gardner, *C. pendulum*, *C. minus* Lindl., *C. roei* Benth. (Houston and Ladd, 2002) and *Hibbertia fasciculata* (Bernhardt, 1986). *Lasioglossum* species have been observed to buzz, *Conostephium roei* (Houston and Ladd, 2002), *Hibbertia stricta* (DC.) F.Muell. (Bernhardt, 1984), *H. fasciculata* DC. (Bernhardt, 1986), *Thelymitra nuda* R.Br. (Bernhardt and Burns-Balogh, 1986), *Melastoma affine* D.Don (Gross, 1993), *Dianella caerulea* var. *assera* R.J.F.Hend. (Bernhardt 1995) and *Tetratheca juncea* Sm. (Driscoll, 2003). *Exoneura* species are buzz-pollinators of *Dianella caerulea* var. *assera* (Bernhardt 1995) and *Tetratheca juncea* (Driscoll, 2003). Gross (1993) observed that bees from the genera *Amegilla*, *Lestis*, *Nomia* and *Xylocopa* were also capable of collecting pollen, via sonication, although they did not always do so. At least nine bee families are known globally to contain buzz-pollinators (Thorp, 2000).

Lasioglossum species and the introduced honeybee collected pollen from *S. propinqua* by scraping rather than sonication. Honeybees are not known to collect pollen by sonication (Thorp, 2000), and they ignored the flowers of *S. incarnata*. Honeybees have also been found to ignore the flowers of the buzz-pollinated *Conostephium pendulum* (Houston and Ladd, 2002) and *Dianella* species (Duncan et al., 2004). The introduced *B. terrestris* is a known buzz-pollinator (Dupont and Olesen, 2006) but was not observed to sonicate either *Sprengelia* species. *Exoneura* species has not been observed to visit *S. propinqua*, although it is present at these sites. *Euryglossa* species and *Leioproctus* species have not been observed at the *S. propinqua* sites and it is currently unknown if their geographic range extends into southwest Tasmania. Of the four native bee genera observed during survey, *Lasioglossum* species and *Exoneura* species are floral generalists but some *Euryglossa* species and *Leioproctus* species are known to be oligolectic (Houston, 2000). In Tasmania, these four bee genera represent important pollinators of a range of plant species, particularly from the Fabaceae and Ericaceae (Hingston collection held at University of Tasmania).

On some occasions pollinator activity was absent at *S. incarnata* even though known buzz-pollinators were scraping pollen from other plants nearby. The absence of pollinator activity on *Conostephium* flowers has also been observed on many occasions (Houston and Ladd,

2002). This could be a result of either pollinators seeking nectar, which is not offered by *Sprengelia*; or perhaps unfavourable climatic conditions for mobilising pollen. In general, foraging bees must either rely on honey reserves available prior to foraging – *A. mellifera* can load up on supplies before leaving the nest - or divide their foraging bouts between nectarless and nectariferous flowers – like the majority of bee taxa (Bernhardt, 1989). In contrast to the polylectic nature of many native bee taxa, *A. mellifera* workers usually collect pure pollen loads (Bernhardt et al., 1984). As the honeybee has a preference for foraging on some native plant species and not others, and is likely to collect pure pollen loads, it has the potential to impact not only on the floral evolution of individual native plant species, particularly those with nectarless flowers, but the entire native flora of Australia. Both *S. incarnata* and *S. propinqua* occur with co-flowering nectar-producing plants and honeybees were prevalent at all study sites; honeybee hives were present at the Tasmanian Wilderness World Heritage Area study sites.

In contrast to the other buzz-pollinated epacrid, *Conostephium*, which has hidden anthers and a pendulous, tubular corolla- *S. incarnata* resembles a 'solanoid' flower. However like *Conostephium*, *S. incarnata* does not have the usual colouration associated with many buzz-pollinated plants - yellow anthers and purple or blue petals (Houston and Ladd, 2002) - instead it has pale anthers and bicoloured white and pink petals. Given that a variety of floral morphologies and stamen arrangements are known to be sonicated, it is possible that the presence of drier (and possibly smaller) pollen enables collection by sonication at *S. incarnata*. It is probable that tacky pollen, such as that of *S. propinqua*, would be difficult to mobilise by sonication. In the Styphelioideae, the pollen of the buzz-pollinated *Conostephium pendulum* was found to be dry as was that of the readily mobilised, wind-dispersed pollen of *Richea procera* (F.Muell.) F.Muell. and *R. sprengelioides* (R.Br.) F.Muell. (Houston and Ladd, 2002; Ladd, 2006). This contrasts with the sticky pollen of the bird-pollinated *Prionotes cerinthoides* (Labill.)R.Br. (Johnson et al., 2010 (Chapter 2)) and the likely mammal-pollinated, *Acrotriche serrulata* R.Br. (Johnson et al., 2011 (Chapter 3)).

Houston and Ladd (2002) observed that the buzz-pollination syndrome was present in phylogenetically separated parts of the Styphelioideae. They confirmed that pollen was collected via sonication from *Conotsephium* in the tribe Styphelieae. Now, we have confirmed that pollen is also collected by sonication from *S. incarnata* in the tribe Cosmelieae. Thus,

there has been independent development of flowers suitable for this form of pollen collection in the Styphelioideae. Although there is currently no phylogenetic hypothesis for the genus *Sprengelia*, it is possible that *S. incarnata* with a floral form suitable for sonication was derived from a *S. propinqua*-type ancestor exhibiting the more common tacky pollen and spreading anthers. The intergradation of floral presentation between these two species could be viewed as supporting evidence for such a hypothesis.

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4.8 Appendix

Appendix 4-1 Tasmanian Herbarium, Hobart (HO) voucher specimens for *Sprengelia*

Sprengelia incarnata 2608, 5168, 5719, 5720, 5721, 5775, 5783, 5801, 5802, 8605, 51876, 72097, 79848, 89782, 94825, 106431, 119960, 400831, 405983, 407896; and *Sprengelia propinqua* 2473, 5757, 5763, 5799, 5804, 58204, 76339, 77618, 89566, 119893, 120776, 120813, 121824, 123685, 315596, 401194, 402889, 403717, 404484, 406328

Chapter 5 Evolution of floral diversity relates to pollinator profiles but not taxonomic divisions in *Richea*

In preparation for submission: **Karen A. Johnson** and James B. Kirkpatrick (XXXX) Evolution of floral diversity relates to pollinator profiles but not taxonomic divisions in *Richea* (Ericaceae).

5.1 Abstract

Premise of the study: The adaptation of floral traits to pollination agents is thought to have played a major role in the morphological diversification of angiosperms. In animal-visited plants the petals provide a visual advertisement to pollinators. However, *Richea* (Ericaceae) exhibit an uncommon form of flowering where the corolla forms an operculum which does not open. Instead, it is shed to expose the reproductive organs. Regardless of this, *Richea* taxa exhibit considerable phenotypic variation, suggestive of differences in signalling to pollinators. Here we report their biotic pollinators, assess the relationships between floral trait profiles and pollinator profiles, and examine floristic diversity in two divergent evolutionary groups.

Methods: We document pollinators and floral features and map them onto a molecular phylogeny. Using non-metric multidimensional scaling and pairwise analyses we explore for alignment between a suite of floral features and pollinator profiles, and compare outcomes across divergent evolutionary groups.

Key results: *Richea* taxa have generalised pollination systems. However, bee and bird groups had the most influence on the ordination of pollinator profiles, and floral trait profiles were associated with pollinator profiles. Although individual floral traits (such as stamen colour) differ between evolutionary groups, the groups show no significant difference between floral trait profiles.

Conclusions: Multivariate analysis revealed floral trait profiles were associated with pollinator profiles but did not reveal distinct floral syndromes between the taxonomic sections of *Richea.* Thus, evolution of floral trait profiles may be occurring in response to pollinators.

5.2 Introduction

The adaptation of floral traits to biotic and abiotic pollination agents is thought to have played a major role in the morphological diversification of angiosperms (Pauw 2006). In some plant genera an array of floral forms have been associated with differences in pollination systems. For instance, "pro-bird" and "anti-bee" adaptations have been shown to be important in shaping floral morphology (Castellanos *et al.* 2004). *Penstemon* flowers vary in their pollination syndromes from those associated with bees and wasps to those associated with hummingbirds (Wilson *et al.* 2006), and plants with a high nectar reward and large floral display are more likely to be visited by hummingbirds than insects (Smith *et al.* 2008). Hummingbirds changed their preference from red to white flowers when the latter offered a better nectar reward (Melendez-Ackerman *et al.* 1997). In *Govenia*, a change from white to yellow flowers, associated with a greater number of flowers on longer inflorescences, is thought to indicate a pollinator shift from small to larger bumblebees (Garcia-Cruz and Sosa 2005).

Richea species exhibit considerable phenotypic variation in their flower morphology. They also exhibit an unusual floral feature, whereby the flower consists of an operculum (cap-shaped corolla) with tiny lobes that generally fail to open (Menadue and Crowden 2000). Instead, the corolla splits transversely near the base and the top part abscises exposing the reproductive organs (Curtis 1963, Walsh and Entwisle 1996). Corolla abscission to present the reproductive organs appears to be a relatively rare phenomenon, with scattered examples in Proteaceae (*Banksia*) and Myrtaceae (*Eucalyptus*, *Melaleuca*, *Leptospermum*, *Callistemon*). The resulting brush-type flowers have been linked to allophilic pollination systems (Hingston and McQuillan 2000). In *Richea*, when numerous opercula are abscised on an inflorescence, the collective presentation of stamens (4-5 per flower) and stigmas bears some similarity to a brush flower. The subfamily Styphelioideae (Ericaceae) is associated with tubular flowers. Thus, *Richea* is unusual for this clade. However, there remains little information on the pollination ecology of *Richea* species.

Richea procera, *R. sprengelioides*, *R. milliganii* and *R. victoriana* have small, dry pollen that can be dispersed by wind (Ladd 2006). Nectaries are absent in *R. procera*, *R. sprengelioides*, and *R. victoriana* (Menadue and Crowden 2000) - another characteristic of wind-pollinated plants (Culley *et al.* 2002; Friedman and Barrett 2008; Friedman and Barrett 2009). Wind-

pollinated plants often have small or no petals, and the use of wind as a pollen vector provides a potential reason to abscise the corolla to increase the efficiency of pollen dispersal (Friedman and Barrett 2009). However, while some *Richea* species use wind as a pollen-vector others lack many of the qualities typical of a wind pollination syndrome, for example, dioecy, plain flowers, feathery styles, and a single ovule (Culley *et al.* 2002; Friedman and Barrett 2008; Friedman and Barrett 2009). Many *Richea* species have sticky pollen that is unsuitable for transport by wind (Ladd 2006). Furthermore, there is some evidence that some *Richea* inflorescences attract animals (Green and Osborne 1994; Corbett 1995; Hingston and McQuillan 2000; Olsson *et al.* 2000).

In animal-visited plants the petals usually provide a visual advertisement. In the absence of petals, plants may need to rely on other forms of advertisement for a nectar reward, such as fragrance or, as in *Banksia* species, brightly coloured reproductive organs. *Banksia ilicifolia* signals its readiness to pollinators with a change in anther colour from red or pink to yellow (Lamont and Collins 2006). Like most of the *Richea* species, the corolla of another epacrid, *Acrotriche serrulata*, is effectively closed distally (McConchie *et al.* 1986), but unlike *Richea*, *A. serrulata* flowers are cryptic and hidden amongst foliage. *Acrotriche serrulata* is known to attract nocturnal mammals, which appear to remove its corolla and may effect pollination (McConchie *et al.* 1986; Johnson *et al.* 2011 (Chapter 3)). Similarly, Olsson *et al.* (2000) suggested that foraging lizards may be required for operculum removal in *R. scoparia* and may, by removing the operculum, facilitate access for insect pollinators.

During the Eocene (over 33 mya) the tribe Richeeae diverged into two lineages – *Sphenotoma*, and *Dracophyllum* plus *Richea* (Wasgstaff *et al.* 2010). Wagstaff *et al.* (2010) have recently found *Richea* and *Dracophyllum* to be paraphyletic. Thus, clades of corolla-abscising taxa occur within a larger group containing flowers which retain their corollas. On the basis of morphological and phytochemical criteria, *Richea* is divided into two sections: section *Cystanthe* (R.Br.)Benth. comprised of *R. acerosa* (Lindl.) F.Muell., *R. milliganii* (Hook.f.) F.Muell., *R. procera* (F.Muell.) F.Muell., and *R. sprengelioides* (R.Br.) F.Muell.; and section *Dracophylloides* Benth. comprised of *R. alpina* Menadue, *R. dracophylla* R.Br., *R. gunnii* Hook.f., *R. pandanifolia* Hook.f., *R. scoparia* Hook.f., *R. continentalis* B.L.Burtt and *R. victoriana* Menadue (Menadue and Crowden 2000; Wagstaff *et al.* 2010). The

molecular phylogeny of Wagstaff *et al.* (2010) provides support for these groups suggesting that section *Cystanthe* formed a well-supported clade that diverged at least 7 mya. Section *Cystanthe* is phylogenetically separated from *Dracophylloides* by a clade of *Dracophyllum,* and *D. minimum* occurs within *Dracophylloides* (Wagstaff *et al.* 2010).

Our aims were to determine the biotic pollinators of the corolla-abscising *Richea* taxa; examine the evolution of floral traits and pollination systems; and explore whether floral trait profiles correlate with pollinator profiles. To determine if floral similarity was related to evolutionary proximity we compared the floral trait profiles of taxa in the two sections that diverged in south eastern Australia at least 7 million years ago: *Cystanthe* and *Dracophylloides*.

5.3 Materials and Methods

5.3.1 Study species and sites

Richea species are small to large shrubs which are restricted to southeastern Australia (Curtis 1963; Menadue and Crowden 2000). They produce small capsules (Menadue and Crowden 2000) with numerous tiny seeds that are most probably dispersed by wind. Our study included the 11 species currently known as *Richea*, except for *Richea curtisiae* which is a hybrid between *R. pandanifolia* and *R. scoparia* (Menadue and Crowden 2000). Nomenclature for vegetation types follows Harris and Kitchener (2005). All study sites were in southern Tasmania. The main study area was Mt. Field National Park with sites for *R. scoparia* (42°39'34"S 146°38'14"E), *R. acerosa* (42°39'46"S 146°38'08"E) and *R. sprengelioides* (42°39'39"S 146°38'57"E) in highland treeless vegetation; for *R. pandanifolia* ssp. *ramulosa* in rainforest/wet eucalypt forest (42°40'56"S 146°35'26"E); *R. milliganii* in wet eucalypt forest-buttongrass moorland ecotone (42°41'55"S 146°34'26"E); and for *R. scoparia* and *D. minimum* in highland vegetation (42°39'29"S 146°31'04"E). The study site for *R. procera* was in wet eucalypt forest in the Wellington Range Park (42°54'24"S 147°15'47"E); and for *Richea dracophylla* was in wet eucalypt forest at Snug Tiers (43°3'59"S 147°10'45"E).

5.3.2 Potential pollinators

We documented the pollinators of each plant species. Consistent with previous published studies (e.g. Ollerton *et al.* 2009), a floral visitor was recorded as a pollinator only after greater than five 'legitimate' visits had been observed. That is, the animal was observed to contact reproductive organs, collect pollen and move between conspecific plants. We also recorded floral visitors that were not observed to make five legitimate visits. We differentiated these two groups (score 2 = > 5 legitimate visits, score 1 = 1 to ≤ 5 legitimate visits). Only animals with greater than five legitimate visits (score 2) were included in our analyses (Appendix 5-1, Table A). Animals were categorised into functional groups based on Fenster *et al.* (2004) – bee (Hymenoptera), wasp (Hymenoptera), fly (Diptera), beetle (Coleoptera), bird (Aves), lizard (Scincidae)).

Over 360 hours of field observations were made on the animal visitors to *Richea* species during their peak flowering times. Observations were made on *R. acerosa* (Jan 2010), *R. dracophylla* (Oct and Nov 2009), *R. milliganii* (Oct and Nov 2009) *R. pandanifolia* (Dec and Jan 2009), *R. procera* (Oct 2007), *R. scoparia* (Jan 2009) and *R. sprengelioides* (Jan 2009). Surveys were conducted between 10.00 and 16.00 hours on fine clear days with little wind (< 3 m per sec). Flowers with available nectar and pollen were chosen for observation in person and by video camera (Panasonic Digital Video Camera, model no. NV-GS70, 1.7 MP, 500x digital zoom and /or JVC Digital Video Camera, model no. GZ-MG465, 1.07 MP, 32x optical zoom) on a tripod. Additional surveys for birds were done between 07.00 and 10.00 hours for *R. dracophylla* and *R. pandanifolia* ssp. *ramulosa* (Nov and Jan 2009). A motion triggered camera (Scout Guard Digital Video Camera, model no. DTC-530V, 5 MP, waterproof and quick response motion triggered PIR (< 1.2 sec)) was used to assist in determining if birds visited *R. dracophylla* and *R. milliganii*.

Samples of the foraging insects were collected by netting or capturing directly into a plastic screw-top container wetted with ethanol. Insects were killed and stored in screw-top vials with 70% ethanol. Bees were identified to genus under a dissecting microscope using the key of Michener (1965) and the Hingston bee collection (housed at the School of Geography and Environmental Studies Laboratory, UTAS) which holds specimens determined by Dr. Ken Walker (National Museum of Victoria). Flies were identified using Colless and McAlpine (1991) and butterflies using Braby (2004). Other invertebrates were identified using

Zborowski and Storey (2003), Daley (2007) and Shattuck (1999). Lizards were identified from close-up photographs using Huchinson *et al.* (2001). Using Pizzey and Knight (1997), bird visitors were identified with binoculars or from close-up photographs and video imagery as they visited flowers. Voucher specimens of potential pollinators collected during surveys are held at the School of Geography and Environmental Studies Laboratory (University of Tasmania).

5.3.3 Floral traits

With the exception of field observations on additional corolla colours for *R. milliganii* (pale green) and *R. pandanifolia* (pink), all information on floral traits and flowering times were gained from the literature (Curtis 1963, Jarman *et al.* 1988, Walsh and Entwisle 1996, Menadue and Crowden 2000, Ladd 2006). We scored the floral traits and flowering times for all *Richea* taxa as presence/absence data. We included all known variability in traits that were described for each taxa. Traits were scored as 1 if present and 0 if not. For instance, corolla abscises closed = 1 or corolla does not abscise closed = 0; orange corolla = 1 or not orange corolla = 0. The other traits scored in this way were: white corolla, pale green corolla, yellow corolla, pink corolla, red corolla, white stamen, yellow stamen, pink stamen, red stamen, flowers nectarless, dry pollen, upright inflorescence, simple inflorescence, hidden inflorescence, pendulous inflorescence, spring flowering, summer flowering, winter flowering, and autumn flowering. Although there are additional traits that could have been included in this study, these traits have all featured in the pollination literature, and together cover most of the floral diversity observed in *Richea*.

5.3.4 Self-pollination experiment

To investigate whether *Richea* species could self-pollinate, we examined seed set in the absence of animals in *R. acerosa, R. scoparia*, and *R. sprengelioides*. For each species, five plants exhibiting budset were chosen. An inflorescences on each plant was bagged with a Terylene mesh bag (approximately 20 cm by 30 cm, with ¼ mm mesh) to exclude animal visitors. The spiky leaves surrounding the inflorescence were trimmed to prevent puncturing of the bag. No other manipulation was undertaken. Capsules were collected prior to dehiscence. Seeds were cut open with a scalpel and assessed for the presence of endosperm under a dissecting microscope. Cut-tests were performed on 686 *R. scoparia*, 365

R. sprengelioides, and 204 *R. acerosa* capsules.

5.3.5 Evolutionary context

We present a phylogenetic tree based on the Bayesian analysis in Fig. 6 (p. 247) of Wagstaff *et al.* (2010). We used the phylogeny to explore the possible evolution of floral traits and potential pollinators (Appendix 5-1). Wagstaff *et al.* (2010) did not include *R. dracophylla* in their molecular analysis. However, morphological and phytochemical analyses have placed it with section *Dracophylloides* (Menadue and Crowden 2000), and we have included it with this grouping for floral trait mapping and analyses.

5.3.6 Data analysis

Using NMDS in the ecodist package in R 2.2.1 (Goslee and Urban 2007) we explored the relationships between all of the *Richea* taxa based on floral traits and flowering times. An ordination was undertaken using all known variation within each plant species, including multiple corolla and stamen colours (Appendix 5-1, Table B). The Bray-Curtis dissimilarity matrix was used as input to the ordination. The attributes that were significantly ($P < 0.05$) linearly related to the variation in species profiles were fitted as vectors. Vectors indicate the direction and strength of the relationships of attributes in ordination space. *P*-values for vectors were based on 1000 permutations. For the purpose of discussion, we have highlighted species representing the evolutionary sections, *Cystanthe* and *Dracophylloides*, on the ordinations.

Using the previously outlined NMDS ordination method on our field work subset of *Richea* taxa (Appendix 5-1, Table A), we explored the relationships between plant species based on their pollinator profiles. An ordination of plants by their pollinators (categorised as the following functional groups – bee (Hymenoptera), fly (Diptera), beetle (Coleoptera), bird (Aves), and wind - was undertaken. Analyses included only those animal groups where at least one animal had been observed to undertake greater than five legitimate visits to a flower (that is, had received a score of 2) (Appendix 5-1 – Table A). Thus, the wasp and lizard categories were not used in the analyses. Furthermore, we only included native pollinators in the analyses. We excluded the one introduced species, the honey bee *Apis mellifera*. We do not consider the honey bee, introduced to Australia in the early nineteenth century, as part of the historical pollinator assemblage as it is unlikely to have had time to effect evolutionary

change on floral traits. The potential pollinators that were significantly ($P < 0.05$) linearly related to the variation in potential pollinator profile were fitted as vectors. *P*-values were based on 1000 permutations.

We undertook an NMDS ordination on our field work subset of *Richea* species based on floral traits and flowering times (Appendix 5-1 – Table B) and the potential pollinators that were significantly ($P < 0.05$) linearly related to the variation in floral trait profile were fitted as vectors.

Using the Mantel test in the ecodist package in R 2.2.1 (Goslee and Urban 2007) and our field work subset of the *Richea* species (Appendix 5-1 – Tables A and B), we tested whether the Bray-Curtis dissimilarity matrix based on pollinators was correlated with the Bray-Curtis matrix based on floral traits. All *P*-values are for the two-tailed test and were based on 10,000 permutations.

To test whether floral traits varied between the *Cystanthe* and *Dracophylloides* groups (Appendix 5-1 – Table 2) we used the Mantel test, as above, to assess whether the Bray-Curtis matrix based on floral traits was associated with a matrix containing 0s for pairs of species within section *Cystanthe* or pairs of species within section *Dracophylloides*, and 1s otherwise.

5.4 Results

5.4.1 Potential pollinators

No flowers were exclusively bird, bee, fly, beetle, wasp or lizard-visited (Table 5-1, Fig. 5-1). Even in the solely-entomophilous species, pollen was dispersed by more than one functional group of insects. Flies and bees accounted for the greatest number of pollinating taxa. Flies visited all of the study species, and with the exception of *R. scoparia*, so did bees. The taxa known to use wind as a pollen vector, *R. procera* and *R. sprengelioides,* also had their pollen dispersed by a range of bees and flies. *Richea pandanifolia* and *R. dracophylla* were visited by birds. The snow skink, *Niveoscincus* sp. foraged on *R. scoparia.* There was considerable overlap in the potential pollinator profiles of the plant species.

Bees, flies and beetles were readily observed, at close range, collecting, transporting, and depositing pollen. Bees were most commonly observed scraping pollen from a flower with their front legs. However, at the flowers of *R. procera* and *R. sprengelioides* they put their legs inside the anthers and scooped the pollen out, and at *R. milliganii* they pressed their bodies against the anthers to collect the pollen. The exposed nature of the reproductive organs, after corolla abscission, facilitated the observation of honeyeater birds (Meliphagidae) undertaking legitimate visits.

Snow skinks (*Niveoscincus* species) climbed all over the flowering plants of *R. scoparia* on the warm still days of the surveys. They removed and consumed operculums, or licked nectar from them, by tearing them apart. They were not observed to damage the reproductive organs during operculum removal. Although they would lick nectar from around the exposed reproductive parts, out of the 30+ corolla removal events observed, fewer than five legitimate visits were recorded. Unlike the insects which carried pollen visible to the naked eye on their heads and bodies, pollen was not obvious on the smooth skin of the skinks during field observation or on close-up photographic footage (although its presence is suspected). They caught and consumed insects while on a plant and tended to stay on the same plant to ambush prey rather than move between plants.

Ants (*Iridomyrmex* species) were seen nectar-robbing on *R. dracophylla* and *R. milliganii*. Geometrid caterpillars took nectar from *R. dracophylla*, grasshoppers (*Russalpia albertisi*) were destructive feeders on *R. milliganii*, and black currawongs (*Strepera fuliginosa*) were destructive feeders on *R. dracophylla.* Shield bugs (Pentatomidae) were observed on *R. milliganii* plants, midges (Ceratopogonidae) and flies (Empididae) on *R. pandanifolia*, and flies (Lauxaniidae) on *R. procera*, but none of these were observed to undertake legitimate visits.

Table 5-1 Potential biotic pollinators of *Richea* species (Am = *Apis mellifera*, Esp = *Exoneura* sp., Lp = *Lasioglossum* (*Parasephecodes*), Lsp = *Leioproctus* sp., Ca = Calliphoridae, He = Heleomyzidae, Tac = Tachinidae, Tab = Tabanidae, Sy = Syrphidae, Ct = *Chauliognathus tricolor*, El = Elateridae, Ic = Ichneumonidae, Het = *Heteropelma* sp., Pe = Pelecorhynchidae, Pp = *Phylidonyris pyrrhoptera*, Lf = *Lichenostomus flavicollis*, Ap = *Anthochaera paradoxa*)

	Bee				Fly						Beetle		Wasp		Bird		
	Am	Esp	Lp	Lsp	Ca	He	Pe	Tab	Tac	Sy	Ct	El	Ic	Het	Pp	Lf	Ap
R. acerosa	1	1	1		1			1		1							
R. milliganii	1	1	1							1				1			
R. procera	1	1			1												
R. sprengelioides	1	1				1				1		1					
R. dracophylla				1						1			1		1		1
R. pandanifolia		1						1		1					1	1	
R. scoparia					1				1		1						

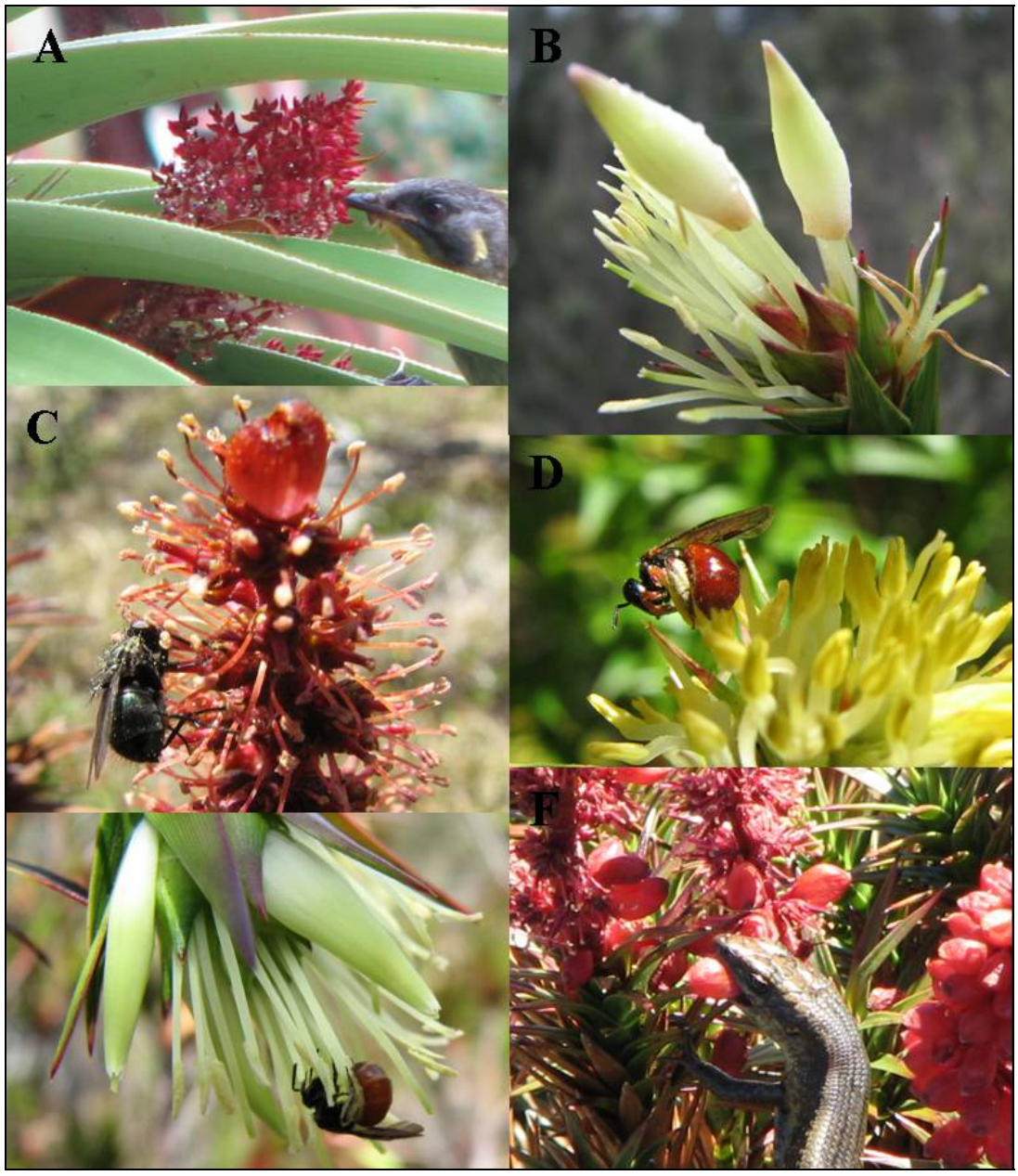

Figure 5-1 A. Yellow throated honeyeater (*Phylidonyris pyrrhoptera*) visiting *R. pandanifolia*; B. Corolla abscission in *R. acerosa*; C. Tachinid fly visiting *R. scoparia*; D. Native bee (*Exoneura* sp.) scraping pollen from *R. procera*; E. *Exoneura* sp. collecting pollen from *R. milliganii*; F. Snow skink (*Niveoscincus* sp.) with operculum it has removed from *R. scoparia.*

5.4.2 Relationships between floral trait and pollinator profiles

In the ordination of all *Richea* species, six of the 20 plant attributes had significant influence on the variation among the *Richea* species: yellow stamen (r = 0.795, *P* = 0.017), pink corolla (r = 0.838, *P* = 0.011), red corolla (r = 0.874, *P* = 0.008), red stamen (r = 0.928, *P* = 0.001), dry pollen (r = 0.797, *P* = 0.015), and inflorescence upright (r = 0.782, *P* = 0.018) (Fig. 5-2). The plant species were scattered, reflecting a general dissimiliarity in floral traits and flowering times. *Richea milliganii* appears to be the most different of the *Richea* species. The vectors for pink corolla, red corolla and red stamen were located close together and away from those for yellow stamen and dry pollen. With the exception of *R. acerosa*, taxa in the *Cystanthe* were associated with the dry pollen and yellow stamen vectors.

Two of the seven animal groups had a significant influence on the ordination of plant pollinator profiles and were described by divergent vectors across the plot - bee (r = 0.964, *P* = 0.009) and bird (r = 0.902, *P* = 0.040) (Fig. 5-3). Taxa in the *Cystanthe* group were loosely clustered in ordination space associated with a greater propensity towards bee pollination (above 0.0 on Axis 1 and below 0.2 on Axis 2). However, we found that no individual animal group had a significant association with floral trait profile.

In contrast, the Mantel test demonstrated that floral trait profiles and pollinator profiles were significantly correlated (r = 0.489, *P* = 0.045). There was no significant relationship between floral trait profiles and the evolutionary groups of *Cystanthe* and *Dracophylloides*.

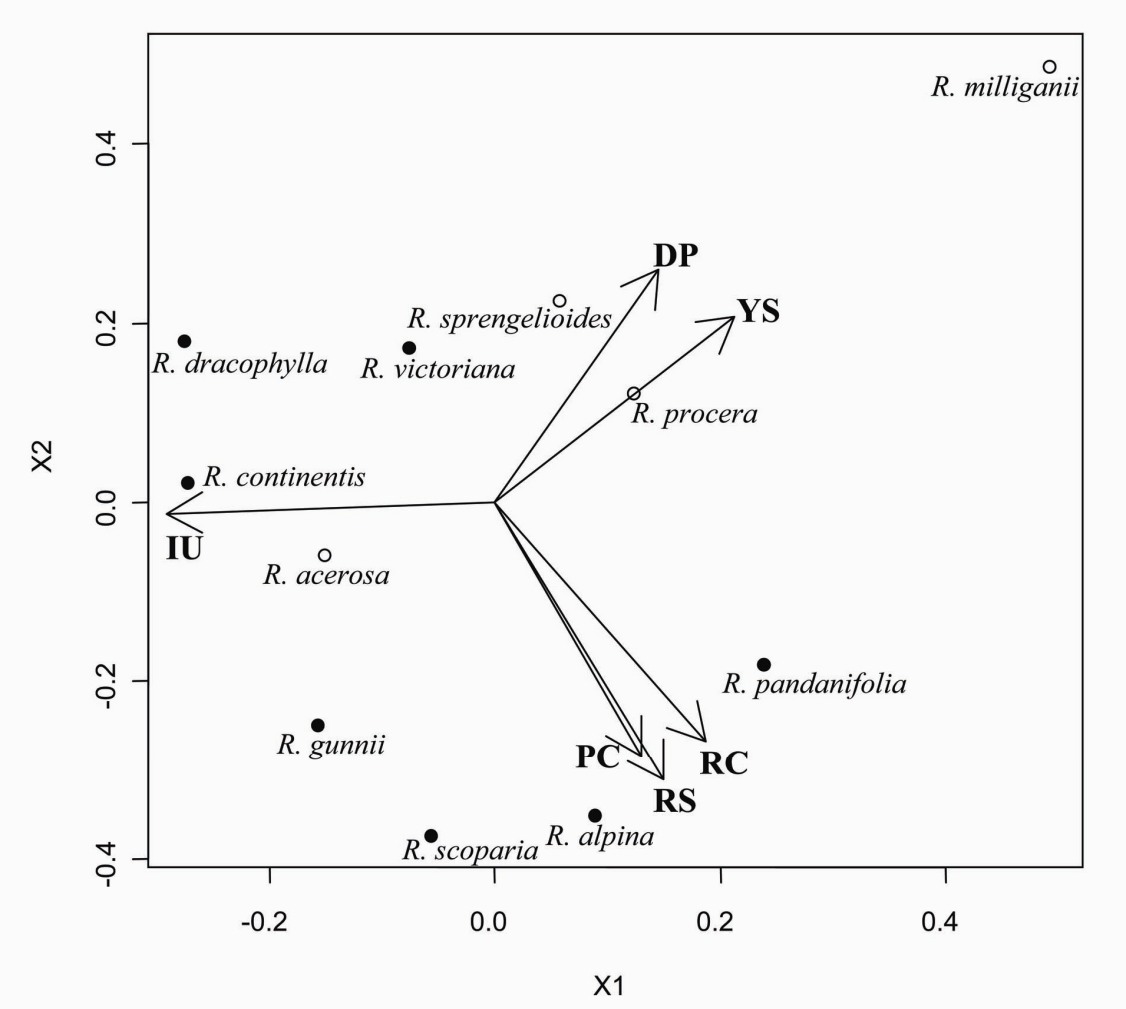

Figure 5-2 Ordination of all *Richea* according to floral trait and flowering time profiles (Dp = dry pollen, YS = yellow stamen; RC = red corolla; RS = red stamen; PC = Pink corolla; IU = inflorescence upright. White dot = members of *Richea* sect. *Cystanthe*, black dot = *Richea* sect. *Dracophylloides*). Attributes that were significant (*P* < 0.05) predictors of the variation between species are fitted as vectors. Stress for 2D ordination = 0.13, r^2 = 0.921.

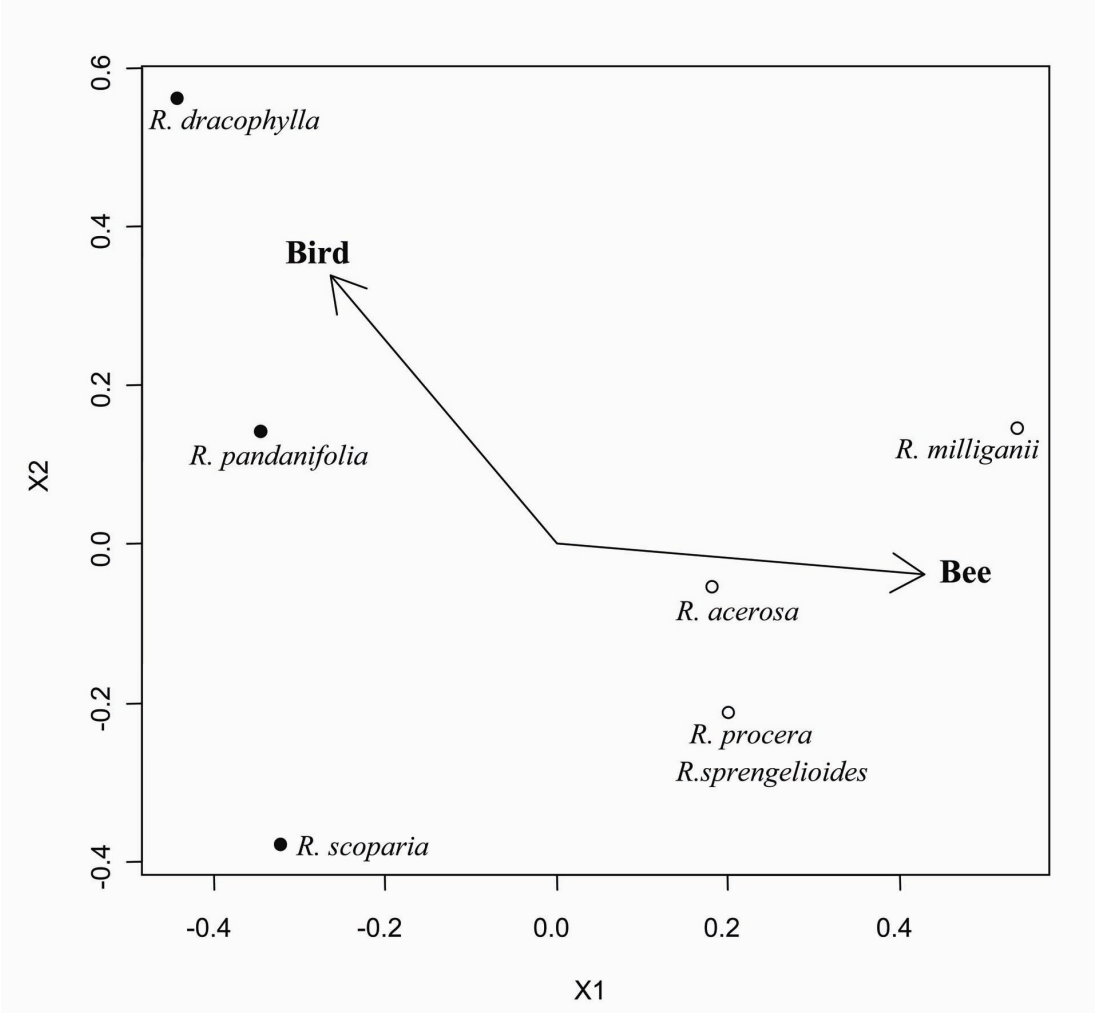

Figure 5-3 Ordination of field work subset of *Richea* according to their potential pollinator profiles (white dot = members of *Richea* sect. *Cystanthe*, black dot = section *Dracophylloides*). Potential pollinators that were significant ($P < 0.05$) predictors of the variation in flower visitor profile are fitted as vectors. Stress for 2D ordination = 0.088, $r^2 = 0.970$.

5.4.3 Self-pollination experiment

The *Richea* species produced capsules in the absence of animal pollinators. Twenty-eight percent of the fruit produced by *R. acerosa*; 19% of fruit produced by *R. sprengelioides*; and 13% of fruit produced by *R. scoparia* contained seed with an endosperm. It was noted that the *Richea* species were able to shed their opercula in the absence of contact by animals.

5.4.4 Evolutionary context

Dry pollen and nectarless flowers are present in taxa from sections *Dracophylloides* and *Cystanthe* (Fig. 5-4). While white stamens are spread across the tree, pink and red stamens are only associated with taxa from *Dracophylloides* and yellow stamens with taxa from *Cystanthe*. *Richea acerosa* is the only taxon from section *Cystanthe* not to have yellow stamens. White and red stamens are basal. White corollas are spread across the tree. Red and orange corollas are associated with taxa from *Dracophylloides* and *R. milliganii* from *Cystanthe* is the only taxon with a yellow corolla. Corolla colour polymorphism is present in nine taxa, and stamen colour polymorphism in two. Only two species show no variability within or between corolla and stamen colour. Bee and fly pollination is prevalent across the tree. Wind pollination occurs in both evolutionary groups but bird pollination has only been recorded in taxa from section *Dracophylloides*.

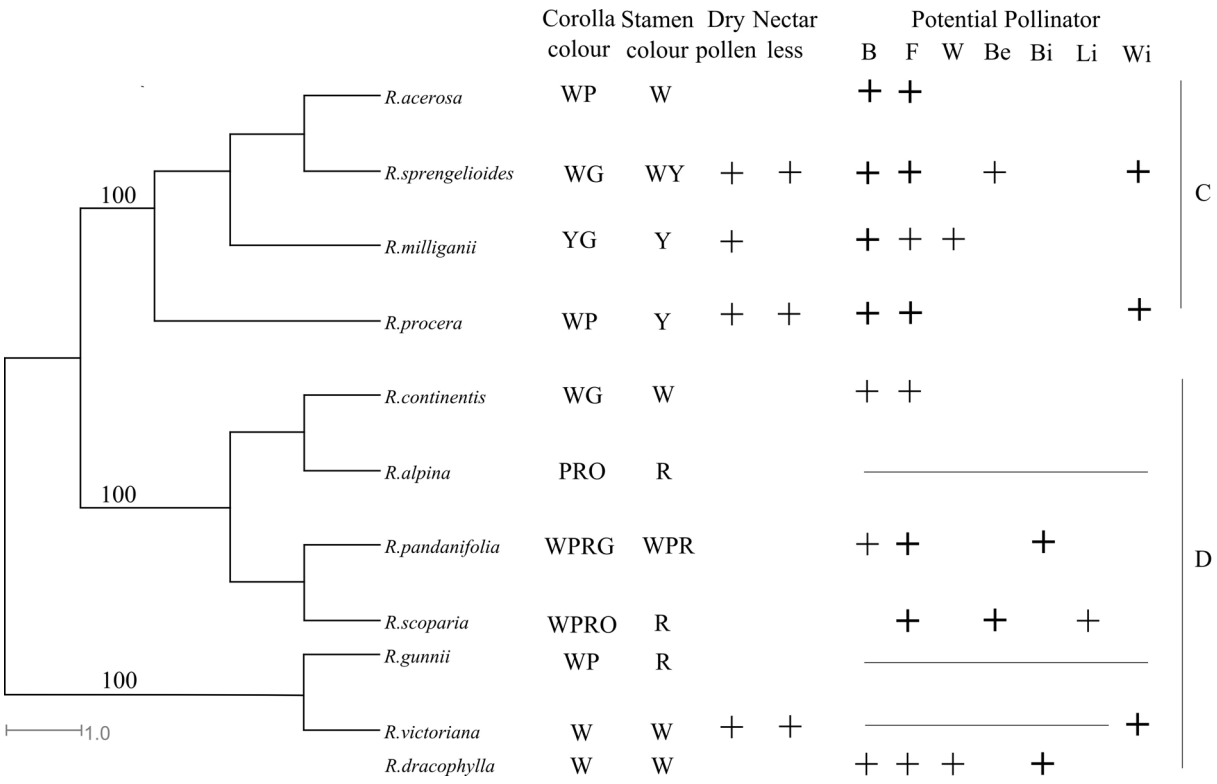

Figure 5-4 Evolutionary mapping of floral traits and pollinators of *Richea* species (Corolla and stamen colour: W = white, P = pink, Y = yellow, R = red, O = orange, G = green. Potential pollinator: B = bee, F = fly, W = wasp, Be = beetle, Bi = bird, Li = lizard, Wi = wind. Bold cross = > 5 legitimate visits observed, Light cross 1 to ≤ 5 legitimate visits observed. C = *Richea* sect. Cystanthe and D = *Richea* sect. Dracophylloides, Line = unknown). Phylogenetic relationships are from the Bayesian analysis in Fig. 6 (p. 247) of Wagstaff *et al.* (2010). Pollinator information for *R. contentis* is from Green and Osborne (1994) and wind pollination is from Ladd (2006)).

5.5 Discussion

Richea flowers fit the profile of allophilic brush flowers, accessible to a range of pollinators including birds and insects. In particular, the different insect groups visited a range of taxa suggesting a lack of pollinator specialisation which is consistent with brush-like flowers in other genera, such as *Eucalyptus, Melaleuca*, and *Leptospermum* (Hingston and Potts 1998; Hingston and McQuillan 2000; Hingston *et al.* 2004). In *Richea*, there were a number of clear divisions between pollinator profiles with *R. milliganii*, *R. procera, R. sprengelioides* and *R. acerosa* (all the members of the *Cystanthe*) associated with bee pollination; and *R. pandanifolia* and *R. dracophylla* with bird pollination. *Richea scoparia* was not associated with either of these significant pollinator groups. It was pollinated by diurnal beetles and flies at both its alpine and subalpine sites. In similar habitats above 700 metres altitude, Hingston and McQuillan (2000) found that flies were the most abundant visitors. This suggests that there may be altitudinal effects on the activity of particular animal groups across southeastern Australia. However, while bees were not observed during the surveys at the alpine site for *R. scoparia* they were present at the subalpine site where they visited the sympatric flowerer, *R. acerosa.* It is not uncommon for epacrid congeners to vary in their visitor profiles at the same site or between sites (Hingston and McQuillan 2000). While bee and bird groups had an influence on pollinator profiles, we found that no individual animal group influenced floral trait profiles, although we did find that the floral trait profiles and pollinator profiles were correlated. For instance, we see from the ordinations that *R. procera*, *R. sprengelioides*, and *R. milliganii* have dry pollen and yellow stamens and are associated with bee pollination. In addition, they both have simple inflorescences; and do not exhibit red or orange corollas, pink or red stamens, autumn flowering, or bird pollination. In contrast, the two bird pollinated species, *R. pandanifolia* and *R. dracophylla*, occupy opposing positions on the floristics ordination. However, they overlap in profiles by having white corollas and white stamens (although corolla and stamen colours in *R. pandanifolia* are polymorphic). Both also have nectaries and complex inflorescences; and do not have orange corollas or yellow stamens, nor are they beetle or wind pollinated.

Unlike the brush-like flowers of *Richea*, the persistent tubular corollas of *Dracophyllum* appear to attract butterflies and moths. In New Zealand, the flowers of *D. acerosum* and *D. uniflorum* are visited by nocturnal moths and craneflies; and *D. pronum* by butterflies and flies (Primack 1983). The Australian endemic, *Dracophyllum minimum*, a sympatric co-flowerer of *R. scoparia*, is pollinated by flies and at least one species of butterfly (*Graphium macleayanus*) (KAJ Jan 2009 unpublished data, recorded in highland vegetation, Mt. Field National Park (42°39'29"S 146°31'04"E)). Thus, the lack of butterfly foraging on *Richea* species is unlikely to relate to habitat or phenology, particularly as most *Richea* taxa flower over summer, the time when most butterflies are likely to be flying (McQuillan 1994). However, consistent with the brush flower syndrome which often includes insects and birds, the two tallest and forest-associated species, *R. pandanifolia* and *R. dracophylla*, include birds in their pollinator profiles (Faegri and van de Pijl 1979). Although we did not examine nectar profiles as part of our study, field observations suggest that *R. pandanifolia*, *R. dracophylla* and *R. scoparia* produce more nectar than the other species. This is likely to relate to their attractiveness to vertebrates and is worthy of investigation. Whether or not lizards act as pollinators is currently uncertain; however, to date they have not been recorded to transport *Richea* pollen between plants (Olsson *et al.* 2000).

The importance of particular floral colours in attracting different pollinators is contentious, but assumptions such as red flowers are bird-pollinated are present throughout the literature (Chittka and Waser 1997, Harrison and Möller 1999, Dressler *et al.* 2004). In *Erica*, it has been suggested that ornithophilous species have become colour polymorphic, and entomophilous ones have speciated (Rebelo and Siegfried 1985). In *Richea*, colour polymorphism is variable between species and commonly occurs within the corolla or between the corolla and stamen. However, most taxa exhibit only one stamen colour. The two bird-visited plants include *R. pandanifolia* which displays colour polymorphism within and between corolla and stamen, and *R. dracophylla* that is unicolourous across both. Likewise, the insect-visited plants include a range of floral colours. Our evidence suggests an association between particular suites of floral characters and suites of pollinators rather than relationships between individual floral attributes and pollinators. It is possible that this reflects the generalised nature of *Richea* pollination systems.

Richea species were visited by animals from at least six groups: bee, fly, wasp, beetle, bird, lizard. Zootic pollen dispersal is common in epacrids (Ford *et al.* 1979; Turner 1982; McConchie *et al.* 1986; Cox 1991; Keighery 1996; Higham and McQuillan 2000; Hingston and McQuillan 2000; Olsson *et al.* 2000; Ladd 2006; Johnson *et al.* 2011 (Chapter 3)), including the most basal extant species *Prionotes cerinthoides* (Johnson *et al.* 2010; Wagstaff *et al.* 2010 (Chapter 2)). Even *R. procera* and *R. sprengelioides,* known to use wind as a pollen vector for their smooth, dry pollen (Ladd 2006), were visited by insects suggesting that they are ambophilous. Attributes including dry pollen and dispensing with nectar are more often associated with wind-pollinated rather than animal-pollinated plants (Friedman and Barrett 2008; Friedman and Barrett 2009). These attributes represent committing floral alterations that have the potential to impact on pollen dispersal by animals. Ambophily may result in greater reproductive success than wind or animal pollination alone, particularly in environments where animal pollinators may be unreliable (Cox 1991). To date, *R. procera* and *R. sprengelioides* are the only species in the Styphelioideae thought to be ambophilic, although *R. victoriana* - nectarless with dry pollen - is also a likely candidate (Ladd 2006). We found *Richea* species to be capable of autonomous-selfing. This facility may also confer reproductive assurance when opportunities for outcrossing are low (Jacquemyn and Brys 2008). Furthermore, it has been discovered that wind-dragged corolla abscission may enhance self pollination (Qu *et al.* 2007). Perhaps, in *Richea*, the closed operculum drags over the reproductive organs, as it abscises, providing the opportunity for the self pollination we observed. Unsurprisingly, this also suggests an environmental influence over the pollination systems of *Richea*.

It is probable that yellow stamens evolved in one evolutionary event in the ancestor of the *Cystanthe* section. Red and pink stamens only occur in taxa from the *Dracophylloides*. However, floral colour polymorphism is spread across the evolutionary tree. In *Richea*, the reproductive organs are only presented after corolla abscission, thus, it is likely that the stamens play a role in indicating reproductive readiness and possibly in attracting pollinators. The adoption of yellow floral parts without corolla abscission has occurred elsewhere, for example, in the New Zealand *Dracophyllum elegatissimum*, in which an anther change from pink to yellow indicates maturity (Venter 2004). In general, the Australian flora has a wide array of yellow-flowered plants associated with animal visitation across the Asteraceae, Dilleniaceae, Fabaceae, Goodeniaceae, Liliaceae, Mimosaceae, and Proteaceae (Hingston and

McQuillan 2000). The classic bee pollination syndrome is most evident in the large, zygomorphic, yellow flowers of the Fabaceae (Faegri and van de Pijl 1979). In contrast, the features relating to bee pollination in *Richea* are more cryptic and relate to a combination of floral characters. In addition, bees (or for that matter, any other animal type) do not represent the sole pollinators of any *Richea* species. It is probable that the diversity of pollen-vectors provides variety to pollen dispersal. For instance, birds and insects may move differently about the landscape and provide different patterns of pollen-dispersal. Furthermore, they may forage under different environmental conditions providing greater robustness to a plant's reproductive efforts.

In conclusion, we found that there were general relationships between floral trait profiles and pollinator profiles, but not between floral trait profiles and taxonomic sections of *Richea.* This latter finding suggests that the evolution of the floral characters reflects a response to pollinator mediated selection.

5.6 Acknowledgements

We thank P. McQuillan for assistance with invertebrate identification and for helpful discussions. We are grateful to S. Wagstaff (Landcare Research, New Zealand) for access to information on the phylogenetic relationships of *Richea* and *Dracophyllum* prior to the recent publication of his manuscript; and to B. Holland (University of Tasmania, Australia) for help using R.

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5.8 Appendix

Appendix 5-1 – Potential pollinators and floral traits of *Richea*

Table A. Potential pollinators of *Richea* species (Biotic groups received a weighting of 2 = at least one taxon from functional group observed to undertake > 5 legitimate floral visits, 1 = at least one taxon from functional group observed to undertake 1 to ≤ 5 legitimate visits. Abiotic vector: 2 = wind pollination possible based on research undertaken by Ladd 2006; note that we have scored *R. milliganii* as 1 in relation to wind pollination. It has dry pollen, however, Ladd 2006 notes that it is different from other wind pollinated species in that it has larger pollen, nectaries and pendulous rather than upright flowers)

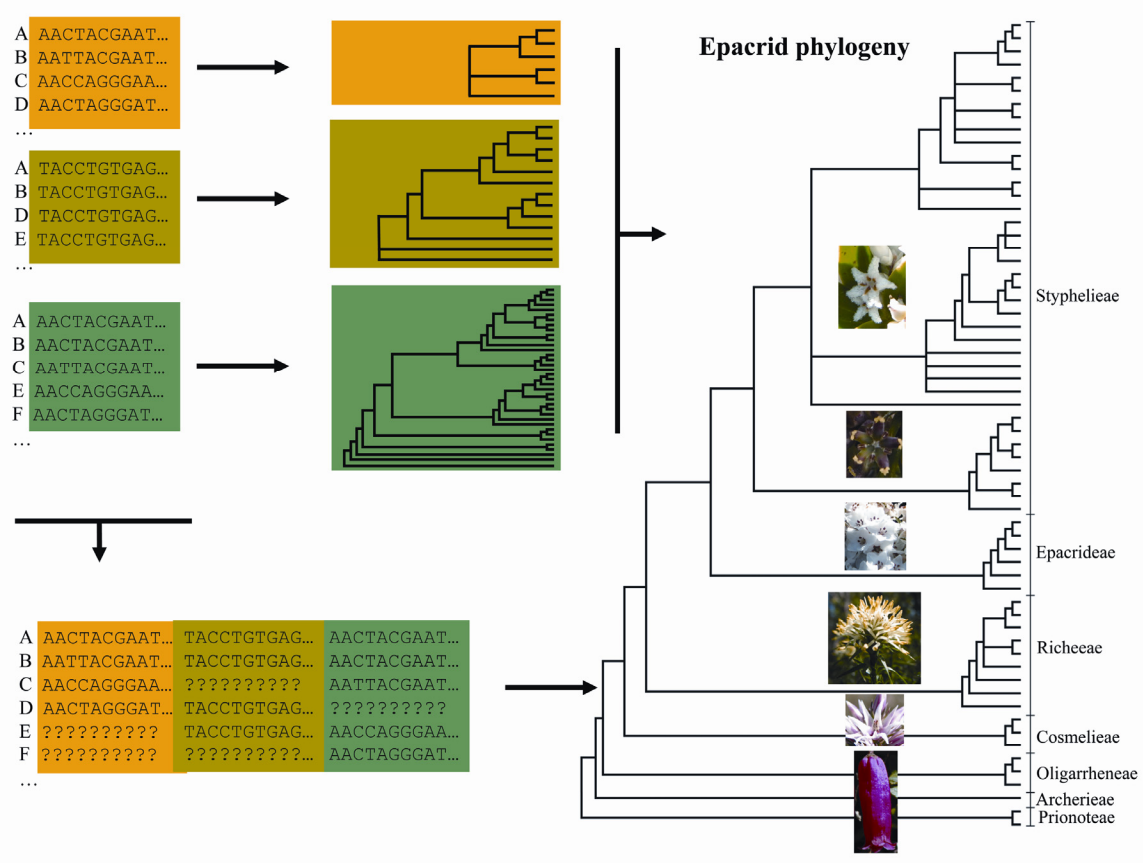
	Bee	Fly	Beetle	Wasp	Bird	Lizard	Wind
R. acerosa	2	2	0	0	0	0	0
R. dracophylla	1	1	0	1	2	0	0
R. milliganii	2	1	0	1	0	0	1
R. pandanifolia	1	2	0	0	2	0	0
R. procera	2	2	0	0	0	0	2
R. scoparia	0	2	2	0	0	1	0
R. sprengelioides	2	2	1	0	0	0	2

Table B. Floral traits and flowering time for all *Richea* species (includes all known variability in traits; Cc = corolla abscises without opening, Co = orange corolla, Cw = white corolla, Cpg = pale green corolla, Cy = yellow corolla, Cp = pink corolla, Cr = red corolla, Sw = white stamen, Sy = yellow stamen, Sp = pink stamen, Sr = red stamen, N = nectarless, Dp = dry pollen, Iu = upright inflorescence, Is = simple inflorescence, Ih = hidden inflorescence, Ip = pendulous inflorescence, FS = spring flowering, FSu = summer flowering, FW = winter flowering, FA = autumn flowering)

	Cc	Co	Cw	Cpg	Cy	Cp	Cr	Sw	Sy	Sp	Sr	N	Dp	Iu	Is	Ih	Ip	FS	FSu	FW	FA
R. acerosa	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	1	0	0
R. dracophylla	1	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0
R. milliganii	0	0	0	1	1	0	0	0	1	0	0	0	1	0	1	0	1	1	0	1	0
R. pandanifolia	1	0	1	1	0	1	1	1	0	1	1	0	0	0	0	1	0	1	1	0	0
R. procera	1	0	1	0	0	1	0	0	1	0	0	1	1	1	1	0	0	1	1	0	0
R. scoparia	1	1	1	0	0	1	1	0	0	0	1	0	0	1	0	0	0	0	1	0	1
R. sprengelioides	1	0	1	1	0	0	0	1	1	0	0	1	1	1	1	0	0	1	1	0	0
R. gunnii	1	0	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0
R. alpina	1	1	0	0	0	1	1	0	0	0	1	0	0	1	0	0	0	1	1	0	0
R. continentis	1	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0
R. victoriana	1	0	1	0	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	0

Chapter 6 Supermatrices, supertrees and the scaffold of serendipity: Inferring a well-resolved phylogeny for the Styphelioideae despite missing data

As submitted (currently under review with Molecular Phylogenetics and Evolution): **Karen A. Johnson**, Barbara R. Holland, Margaret M. Heslewood and Darren M. Crayn (XXXX) Supermatrices, supertrees and the scaffold of serendipity: Inferring a well-resolved phylogeny for the Styphelioideae (Ericaceae) despite missing data.



Graphical abstract

6.1 Abstract

For the predominantly southern hemisphere plant group Styphelioideae (Ericaceae) published sequence data sets of five markers are now available for all except one of the 38 recognised genera. However, several markers are highly incomplete, therefore missing data is problematic for producing a genus level phylogeny. We explore the relative utility of supertree and supermatrix approaches for addressing this challenge, and examine the effects of missing data on tree topology and resolution. Although the supertree approach returned a more conservative hypothesis, overall, both supermatrix and supertree analyses concurred in the topologies they returned. Using multiple genes and a data set of variably complete taxa we found improved support for the monophyly and position of the tribes and genus level relationships. However, there was mixed support for the Richeeae tribe appearing one node basal to the Cosmelieae tribe or vice versa. It is probable that this will only be resolved through further gene sequencing. Our study supports previous findings that the amount of data used is more critical than the completeness of the dataset in estimating well-resolved trees. Our results suggest that a "serendipitous" scaffolding approach that includes a mixture of well and poorly sequenced taxa can lead to robust phylogenetic hypotheses.

6.2 Introduction

Phylogenies provide the context for interpreting the evolution of plant and animal lifestyles, physiologies, morphologies, pollination systems, and geographical radiations. While the ideal phylogenetic hypothesis would be based on a complete data set and resolve higher-level and species-level relationships simultaneously, for many groups a useful strategy is to undertake analyses of novel combinations of existing data. For the predominantly southern hemisphere plant group Styphelioideae Sweet (Ericaceae Juss.), palaeontological, evolutionary and ecological studies can be best facilitated in the near term by exploiting the wealth of existing data to produce a genus level phylogeny using supertree and supermatrix methods.

Styphelioideae (syn. family Epacridaceae R.Br.) comprises 38 genera and more than 550 species of woody plants ranging from small prostrate shrubs to cool temperate rainforest emergents. They are usually found on nutrient poor soils where their capacity to form ericoid mycorrhizae likely contributes to their success. Their range extends from SE Asia through Oceania to Tierra del Fuego but most of the species and the taxonomic diversity in the subfamily is found in Australia, particularly the southwest and southeast. Other centres of diversity occur in New Zealand and New Caledonia. Since the 1990's, phylogenetic relationships of Styphelioideae have received much attention. Molecular and morphological work has improved our knowledge of higher-level relationships in the Ericaceae (Kron, 1996; Powell et al., 1996; Kron et al., 1999; Kron et al., 1999a; Kron et al., 2002) and the monophyly of the Styphelioideae and seven tribes is well established on the basis of DNA sequence data (Crayn et al., 1996; Crayn et al., 1998; Kron, 1996; Kron et al., 1999a; Quinn et al., 2005). Published sequence data are available for all except one (*Decatoca* F.Muell.) of the 38 recognised genera and for many of these genera data from multiple loci are available (Kron and Chase 1993; Kron 1996; Crayn et al. 1996; Crayn et al. 1998; Kron et al. 1999a; Taaffe et al., 2001; Crayn and Quinn 2000; Quinn et al., 2003; Albrecht et al. 2010; Wagstaff et al., 2010).

It is now feasible to pool these data to produce a well-resolved genus-level phylogeny of the Styphelioideae. Wiens et al. (2005) demonstrated using the speciose (> 800 spp.) treefrog family Hylidae that it is possible to resolve higher-level phylogenetic relationships by constructing a "scaffold" from large numbers of taxa with data for slow-evolving sites then

placing onto this "scaffold" taxa for which the only data is a smaller number of more rapidly evolving sites. This enables optimal resolution without requiring data for every character (Wiens, 2006). Pirie et al. (2008) also used the "scaffold" approach to overcome the limitations of previous phylogenetic analyses of danthonoid grasses – namely, insufficient character and taxon sampling. Although the "scaffold" approach results in a matrix dominated by missing data cells, Wiens et al. (2005) found no relationship between a taxon's completeness and support for its localised placement on the tree. So, while the distribution of missing data associated with such an approach is likely to be non-random it may provide an opportunity to build well-sampled and potentially well-resolved trees. In this study we utilise both of the two main approaches for analysing multi-gene datasets with a high proportion of missing data: supermatrix analysis and supertree analysis. In essence, the supermatrix approach analyses all gene data from all taxa simultaneously, while the supertree approach involves the separate analyses of each gene dataset and the subsequent integration of the resulting trees (Sanderson et al., 1998; Bininda-Edmonds, 2004; de Queiroz and Gatesy, 2006). In a standard supermatrix analysis all genes are concatenated, missing data are coded as a "?" and the matrix analysed under a single model of nucleotide substitution (e.g. GTR + I + G). The dangers of this approach are that different genes may evolve under different processes. By forcing one model to apply to all genes the model may fail to capture relevant features of the real process that produced the data, and thereby introduce systematic phylogenetic error which may be hard to detect (Goremykin et al., 2005; Phillips et al., 2004).

Ren et al. (2009) propose what they call a "likelihood approach" which could be thought of as a supermatrix with a partitioned model (Yang, 1996; Pupko et al., 2002). Here different genes are allowed to take on their own model parameters, for instance both MrBayes (Huelsenbeck and Ronquist, 2001) and RAxML (Stamatakis et al., 2008) allow user-defined partitions. In a natural extension of this idea, both MrBayes and RAxML allow that edge lengths can be unlinked between partitions; this means that the only thing that is shared across partitions is the underlying tree topology. This model represents a more biologically realistic situation where different genes are under different constraints in different species (Lopez et al., 2002). However, these models run into problems in the supermatrix setting, in which information about some genes is completely missing for some taxa. In this case the software has no information to use when estimating gene-specific branch lengths for taxa where data for that gene are not present. Lemmon et al. (2009) found that in ML and Bayesian analyses, among-

site variation can interact with missing data and give misleading tree topologies and branch lengths. They also found that the effects of missing data are exacerbated in the Bayesian framework. Although incomplete taxa have been assumed problematic because of the amount of missing data (Rowe 1988; Huelsenbeck 1991; Ebach and Ahyong, 2001) it is likely that inaccuracies are a result of taxa having too few complete sites rather than too many missing sites (Anderson, 2001; Kearney, 2002; Wiens, 2003).

The supertree approach circumvents the problem caused by missing data by analysing each gene completely separately. With this method even the tree topology is free to vary between the genes. The resulting trees are then assembled using a supertree method to give a single phylogenetic hypothesis. The supertree method that is most widely used is matrix representation with parsimony (MRP) (Baum, 1992; Ragan, 1992; Bininda-Edmonds, 2004). The main criticism of the supertree approach is its loss of contact with the primary character data which may lead to pseudoreplication and as a result, a loss of data independence, but this is not a problem if each gene is analysed separately (Kennedy and Page, 2002; Bininda-Edmonds, 2004). Another drawback of this approach is that it is not immediately obvious how to get a measure of support for individual edges, although researchers have been addressing this issue (Bininda-Edmonds, 2003; Wilkinson et al., 2005; Burleigh et al., 2006). Originally the supermatrix and supertree approaches were regarded as competing strategies, however, it has more recently been proposed that these philosophically different methods be used in a complementary fashion to interpret phylogenetic hypotheses (Bininda-Edmonds et al., 2003; Gatesy et al., 2004; Baker et al., 2009). For instance, in analyses where both methods concur then this corroboration engenders a greater degree of confidence in the resulting phylogenetic relationships while points of disagreement may indicate areas where closer scrutiny should be applied (Bininda-Edmonds, 2004; Baker et al., 2009).

Our aim was to obtain a well-resolved genus level phylogeny of the Styphelioideae using existing data. We examined the utility of the "scaffold" concept and the effects of missing data on tree topology and resolution in achieving our aim. We compared the hypotheses from the philosophically different supermatrix and supertree analyses. We expected that the supermatrix approach would have more power to resolve relationships than the more conservative supertree approach. (As the supermatrix approach works directly from the character data it has the potential to reveal character support for relationships in the overall

tree, that are not supported in isolation; and it can also support relationships that are contradicted by separate analyses of those data (de Queiroz and Gatesy, 2006)). Fitting models with a single set of branch lengths to data that has arisen on partitions with different branch lengths can cause systematic error (Kolacziwski and Thorton, 2004). With this in mind, we used the supertree approach as a check on potential systematic error involved in forcing equal branch lengths across all genes in the supermatrix approach.

6.3 Methods

6.3.1 Datasets and Sequence Alignment

A data set of Styphelioideae taxa with sequences for *rbc*L, *mat*K, *atp*β-*rbc*L intergenic spacer, ITS and 18S was constructed from data available from GenBank, Wagstaff et al. (2010), and unpublished data from colleagues. GenBank was searched for all genera enumerated in Stevens (2004), taking account of known taxonomic changes (e.g. Albrecht et al. 2010). We included a provisional (undescribed) genus, '*Pseudactinia*' (Western Australian Herbarium 1998). No sequence data (or material for sequencing) was available for the Papua New Guinea endemic monotypic genus *Decatoca*. Details of GenBank accessions for sequences are given in Appendix 6-1.

To produce our phylogenetic hypotheses we analysed four datasets (Table 6-1). These datasets also provided the framework for examining the effects of taxon and site sampling on tree topology and resolution. We either used a large data set (111 taxa) or a small data set (62 taxa) (Appendix 6-1). The large data set generally included at least two taxa from each genus, plus taxa representative of all areas of known remaining paraphyly (recent work in the *Cyathodes* and *Monotoca* groups have established monophyletic taxa for these formerly paraphyletic assemblages (Quinn et al. 2005; Albrecht et al. 2010)): *Astroloma* R.Br., *Brachyloma* Sond., *Leucopogon* R.Br., *Lissanthe* R.Br., *Styphelia* Sm. (Taaffe et al. 2001; Quinn et al. 2003), *Dracophyllum* Labill., *Richea* R.Br. (Wagstaff et al., 2010), and *Epacris* Cav. (Crayn and Quinn, 2000). It consisted of roughly 20% of the approximately 550 species in the Styphelioideae.

Table 6-1 Data sets

Data set	Taxa no.	Total Sites	Total number of non-missing characters	% of missing characters
5 gene, small	62	6,603	173,689	58%
5 gene, large	111	6,711	256,590	66%
3 gene, small	62	4,114	161,362	37%
3 gene, large	111	4,219	240,885	49%

We undertook a preliminary RAxML analysis of our large data set (111 taxa). Where taxa formed genus-level clades which were consistent with past phylogenetic analyses (Crayn et al., 1998; Kron et al., 1999a; Taaffe et al., 2001; Quinn et al., 2003; Quinn et al., 2005), the taxon with the most complete data across the genes was chosen to represent the genus in the small data set analyses. Any taxon whose position was unexpected was also included. The small data set contained the minimum taxa possible to represent each genus and known paraphyly. It consisted of about 11% of taxa in the Styphelioideae. For each taxon we either used all five genes (*rbc*L, *atp*β-*rbc*L intergenic spacer, *mat*K, 18S, and ITS) if those data existed, or the three genes for which the most data was available (*rbc*L, *mat*K, *atp*β-*rbc*L intergenic spacer), thus creating a large five gene data set, a large three gene data set, a small five gene data set, and a small three gene data set. Appendix 6-1 details the sequences employed for each taxon.

Each gene was aligned separately using MUSCLE (Edgar 2004). Individual alignments were concatenated to form supermatrix alignments using a Python script (available from BRH on request). The Prionoteae (*Prionotes* and *Lebetanthus*) was used as outgroup (except in the single-gene ITS analysis where there was no sequence data for the Prionoteae) since it is monophyletic (Crayn et al., 1998) and robustly placed as sister to the rest of the Styphelioideae (Powell et al., 1996; Crayn et al., 1998; Kron et al., 1999a).

6.3.2 Single Gene Analyses

An appropriate evolutionary model for each gene was chosen using MrModelTest under the Akaike information criterion (Nylander 2004). The models selected for the small (62) data sets were *rbc*L GTR + I + G, *mat*K and *atp*β-*rbc*L intergenic spacer GTR + G, and for 18S

and ITS GTR + I. The models selected were the same for the large (111) data sets with the exception of ITS where GTR + G was selected. To determine the likely effects of individual genes on our supermatrix and supertree analyses we estimated the maximum likelihood tree for each gene using the models given above. These analyses were done for both the large (111) and small (62) taxon sets, although obviously for each gene different subsets of these were available (see Appendix 6-1). The RAxML webserver (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis et al., 2008) was used to analyse all alignments.

6.3.3 Supermatrix Analyses

We made a supermatrix of each of the four data sets described in Table 6-1. The five gene, large data set (111 taxa) had 6711 sites that contained *atp*β-*rbc*L intergenic spacer (103 taxa and 1202 sites), *mat*K (87 taxa and 1589 sites), *rbc*L (36 taxa 1428 sites), ITS (14 taxa and 685 sites), and 18S (6 taxa and 1805 sites). The five gene small data set (62 taxa) had 4114 sites that contained *atp*β-*rbc*L intergenic spacer (56 taxa and 1130 sites), *mat*K (55 taxa and 1556 sites), *rbc*L (34 taxa and 1428 sites), ITS (8 taxa and 684 sites), and 18S (five taxa and 1805 sites). Each of the large and small data sets also had a three gene version containing *atp*β-*rbc*L intergenic spacer, *mat*K, and *rbc*L.

An unpartitioned model for each supermatrix was chosen using MrModelTest under the Akaike information criterion (Nylander 2004). The model selected was GTR + I + G in all four cases. The RAxML webserver (http://phylobench.vital-it.ch/raxml-bb/) (Stamatakis, Hoover, and Rougemont 2008) was used to analyse all four supermatrices. MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was also used to analyse the three and five gene supermatrices based on the small data sets. Bayesian analysis of the large data sets was computationally prohibitive.

We performed likelihood analysis in RAxML using either a partitioned or an unpartitioned (GTR + I + G) model. In RAxML the model partitions must all be of the same kind so we used GTR + I + G as this was the most general model found to be appropriate in the single gene analyses. Each gene was allowed its own parameters (including GTR rate matrix parameters, the shape parameter for rates across sites, and the proportion of invariant sites). For reasons outlined in the introduction, we used fixed branch lengths across our partitioned models. We performed Bayesian Monte Carlo Markov Chain (MCMC) analyses in Mr Bayes.

We used a partitioned model that allowed each gene to use the model preferred by MrModelTest in the single gene analyses. The rate matrix parameters, shape parameter and proportion of invariant sites were all unlinked across the partitions. MrBayes was run for 2x5,000,000 generations, sampling every 10,000th tree, with a 10% burn-in. Convergence was assessed by checking that the standard deviation of split frequencies between the two runs was less than 0.02. The two runs were combined for analysis of the results.

6.3.4 Supertree Analyses

We applied supertree methods to the three and five gene small data sets only. We avoided data replication (Bininda-Edmonds 2004) in our analyses by building individual gene trees and then combining them, thus all data was only used once. We used Matrix Representation with Parsimony (MRP) and the standard Baum Ragan coding (Baum 1992; Ragan 1992) of the five ML trees from each of the single gene RAxML analyses (143 characters). Matrix encoding of the trees was performed using Clann version 3.0.0 (Creevey and McInerney, 2005). The resulting matrix was analysed using the heuristic search option in PAUP* version 4.0b10, and a strict consensus of all equally parsimonious trees was formed. To assess support for edges in the resulting supertree we used the hierarchical bootstrapping method proposed by Burleigh et al. (2006). For each of the five genes we constructed a file with 100 bootstrap trees (from the RAxML analyses). We created 100 supertree bootstrap replicates by sampling one tree at random (with replacement) from each of the five RAxML bootstrap files. MRP was applied to each of the resulting 100 sets of five trees. (Python script for performing sampling and producing PAUP* blocks is available upon request from BRH). The results were visualised using majority-rule consensus and consensus networks (Holland et al. 2004) methods as implemented in SplitsTree4 (Huson and Bryant, 2006).

6.3.5 Comparing supermatrix and supertree approaches

To determine if there were any contradictory splits that received posterior probability of > 0.5 or bootstrap support of > 50% in the supermatrix and supertree phylogenetic hypotheses, we used consensus networks as implemented in SplitsTree4 to compare the majority-rule trees formed from the MrBayes posterior distributions, RAxML bootstrap trees, and the MRP hierarchical bootstrap trees.

6.3.6 Effects of taxon and site sampling on resolution

To test the utility of the "scaffold" concept we compared the four data sets (Table 6-2). The datasets differed with respect to the proportion of missing data and the number of non-missing characters (Table 6-1). As previously described we used RAxML to estimate ML trees and bootstrap support for each of the datasets. As our analyses suggested that whether data was partitioned (by gene) or not, made little difference to the resulting tree topology (see results), we used the partitioned GTR + I + G results to explore the effects of taxon and character sampling on resolution. As it is difficult to compare trees of different sizes and because we were most interested in resolving genus level relationships, we pruned the bootstrap trees from each of the analyses on the large data sets (111 taxa) so that they only contained the same 62 taxa as found in the small data sets. This was done using the filter taxa option in SplitsTree4 (Huson and Bryant, 2006). To see how well the different data sets resolved the genus level relationships, we summed the bootstrap support for all splits (edges) that had greater than 50% support in each of the four data sets. There are 59 non-trivial splits in a fully resolved 62 taxon tree so the maximum possible score is 5900. We performed a similar analysis to the one outlined above to compare the results of the three and five gene small data set analyses conducted in MrBayes, to test the effect of character sampling and missing data on resolution in a Bayesian framework.

Table 6-2 Completeness of taxa in each data set

Data set	Total taxa	No. taxa 5 genes	No. taxa 4 genes	No. taxa 3 genes	No. taxa 2 genes	No. taxa 1 gene
5 gene, small	62	0	5	26	29	2
5 gene, large	111	0	5	30	59	17
3 gene, small	62	-	-	23	37	2
3 gene, large	111	-	-	23	69	19

6.4 Results

In the discussions below we use the first letter to represent each tribe (P = Prionoteae, A = Archerieae, O = Oligarrheneae, C = Cosmelieae, R = Richeeae, E = Epacrideae, S = Styphelieae).

6.4.1 Single Gene Analyses

Generally, the single *rbc*L and *mat*K analyses returned the monophyly of most tribes but the relationship between tribes or genera received little support. The *atp*β-*rbc*L intergenic spacer did not resolve all of the traditional tribes but it gave higher support to some genus-level relationships than *rbc*L or *mat*K. There was insufficient information to examine the ITS and 18S genes at the tribe-level. As single gene phylogenies have appeared elsewhere we do not show them here, but have instead collected them in a supplementary file (given at end of chapter in Supplementary 6-1 to 6-5). The *rbc*L analysis showed the following arrangement of tribes: (P,(A,(O,(C,(R,(E,S)))))). However, with the exception of the sister grouping of the Epacrideae and Styphelieae (bootstrap 77% for small data set and 90% for large data set available taxa) the relationships between the tribes were weakly supported. The *mat*K analysis showed the following arrangement of tribes: (P,(A,(O,(R,(C,(E,S)))))). However, there was little support for these positions, the only tribal relationships with > 60% support were the splits PAO | RCES (66%) and PAOR | CES (62%). The *atp*β-*rbc*L intergenic spacer analysis produced low support for the tribes. Richeeae and Oligarrheneae were both paraphyletic. The sister grouping of Epacrideae and Styphelieae received low support (bootstrap 69% for small data set and 63% for large data set).

6.4.2 Supermatrix Analyses

All multiple-gene analyses showed a marked increase in support for tribe and genus-level positions from that of the single-gene analyses. Overall, the Bayesian posterior probabilities tended to be higher than the RAxML bootstrap values. All the analyses supported the monophyly of the tribes with 100% bootstrap support and posterior probability of 1, with the exception of the RAxML analyses where Oligarrheneae had bootstrap support of 95% (five gene pruned large data) and 88% (five gene small data). The following two hypotheses for the tribal relationships received support: (P,(A,(O,(R,(C,(E,S)))))) and (P,(A,(O,(C,(R,(E,S)))))). Table 6-3 compares the support given by each method to the relationships between the tribes. Across the eight RAxML analyses (partitioned or unpartitioned , three or five gene, large or small taxon sets) and the two MrBayes analyses (three or five gene) support for the two possible tribal-level trees is close to 50/50. We found little supported conflict between the various supermatrix analyses (Figs. 6-1, 6-2), i.e. there were very few conflicting splits that both received more than 50% bootstrap support or 0.5 posterior probability. Exceptions were the conflict over the placement of the Richeeae and Cosmelieae tribes described above and in Table 6-3, and the position of both *Cyathopsis albicans* and *Agiortia cicatricatus* for which there were alternative placements, each separated by a single edge (Fig. 6-1, Table 6-4).

Within Oligarrheneae, *Needhamiella* R.Br. is sister to *Dielsiodoxa* Albr. and *Oligarrhena* R.Br. which are robustly grouped (Figs. 6-1, 6-2). This relationship was also supported by *mat*K and *atp*β-*rbc*L intergenic spacer single gene analyses. Relationships within Cosmelieae are well-supported with *Cosmelia* R.Br. and *Sprengelia* Sm. as sister genera (Figs. 6-1, 6-2). Within Richeeae, *Sphenotoma* is sister to a clade comprising *Richea* Labill. and *Dracophyllum* Labill. with high support in the RAxML analyses (Fig. 6-1) but lower support in the Bayesian analyses (Fig. 6-2). The positions of genera in the Epacrideae are well-supported. *Woollsia* F.Muell. is well-supported as sister to the rest of the tribe (Figs. 6-1, 6-2). *Budawangia* I.Telford is sister to *Rupicola* Maiden & Betche (also present in the *atp*β-*rbc*L intergenic spacer analysis). Relationships within the Styphelieae are generally less resolved. However, the areas of conflict are localised (Fig. 6-3). Approximately two thirds of the edges within this tribe received bootstrap support > 50% or posterior probability > 0.5 (Figs. 6-1, 6-2). A number of well-supported groups and genus-level associations are apparent.

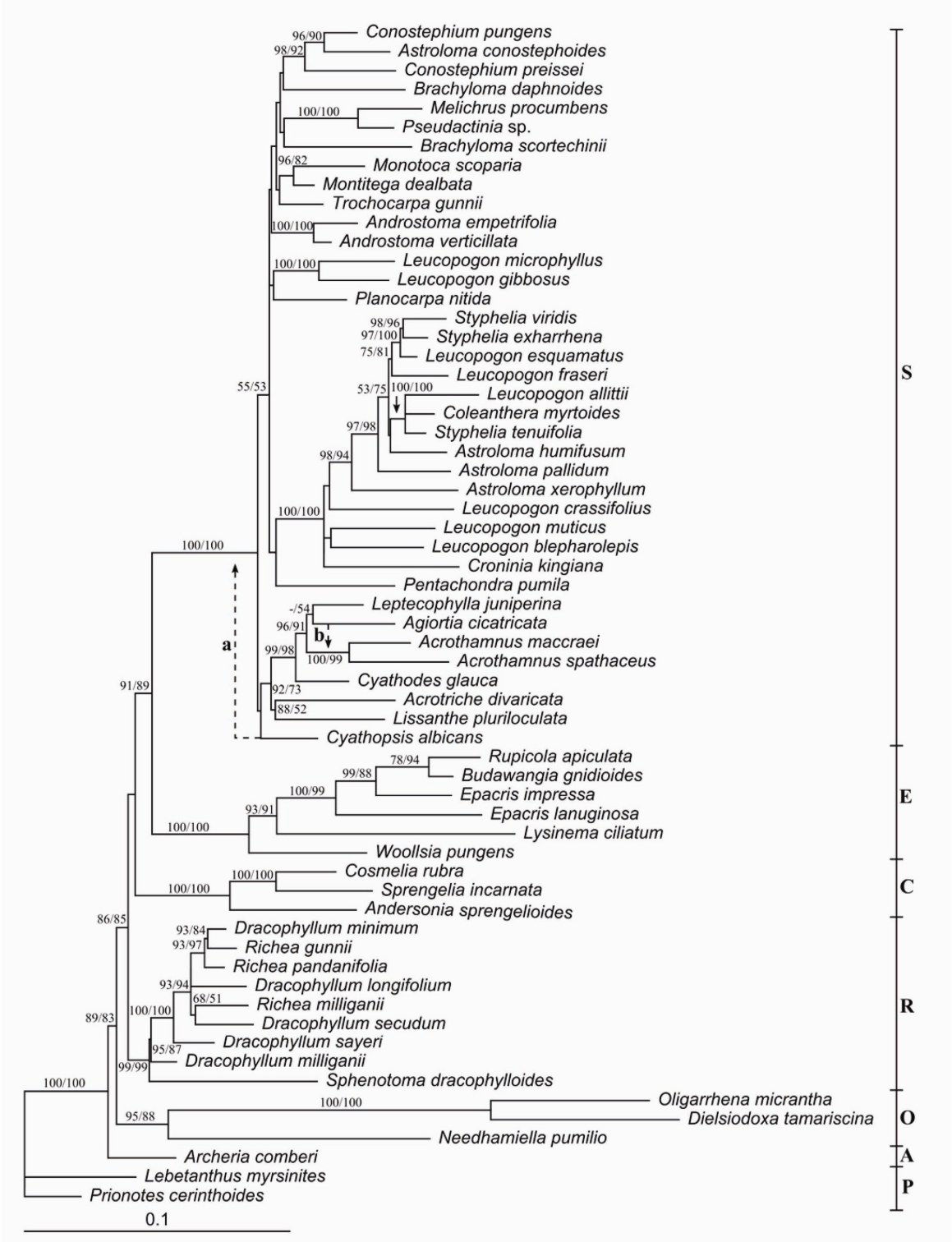


Figure 6-1 RAxML tree (based on five gene pruned data set with splits > 50% annotated with bootstraps from: large pruned data set / small data set; position of tribes annotated: P = Prionoteae, A = Archerieae, O = Oligarrheneae, C = Cosmelieae, R = Richeeae, E = Epacrideae, S = Styphelieae (a = alternative position of *Cyathopsis*, b = alternative position of *Agiortia*).

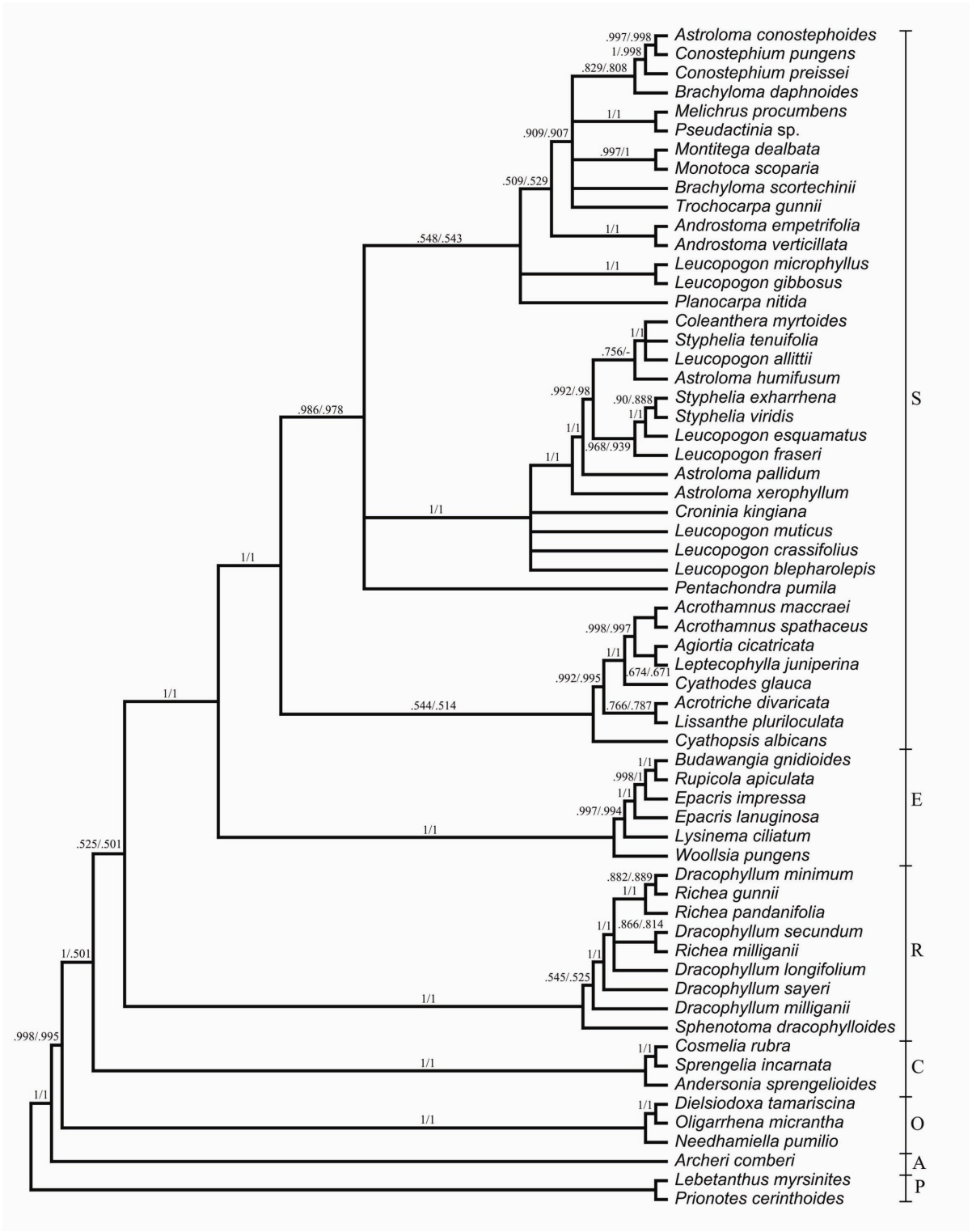


Figure 6-2 MrBayes majority rule tree (based on five gene small data set with splits > 0.50 annotated with posterior probabilities for five gene small data set / three gene small data set; position of tribes annotated: P = Prionoteae, A = Archerieae, O = Oligarrheneae, C = Cosmelieae, R = Richeeae, E = Epacrideae, S = Styphelieae).

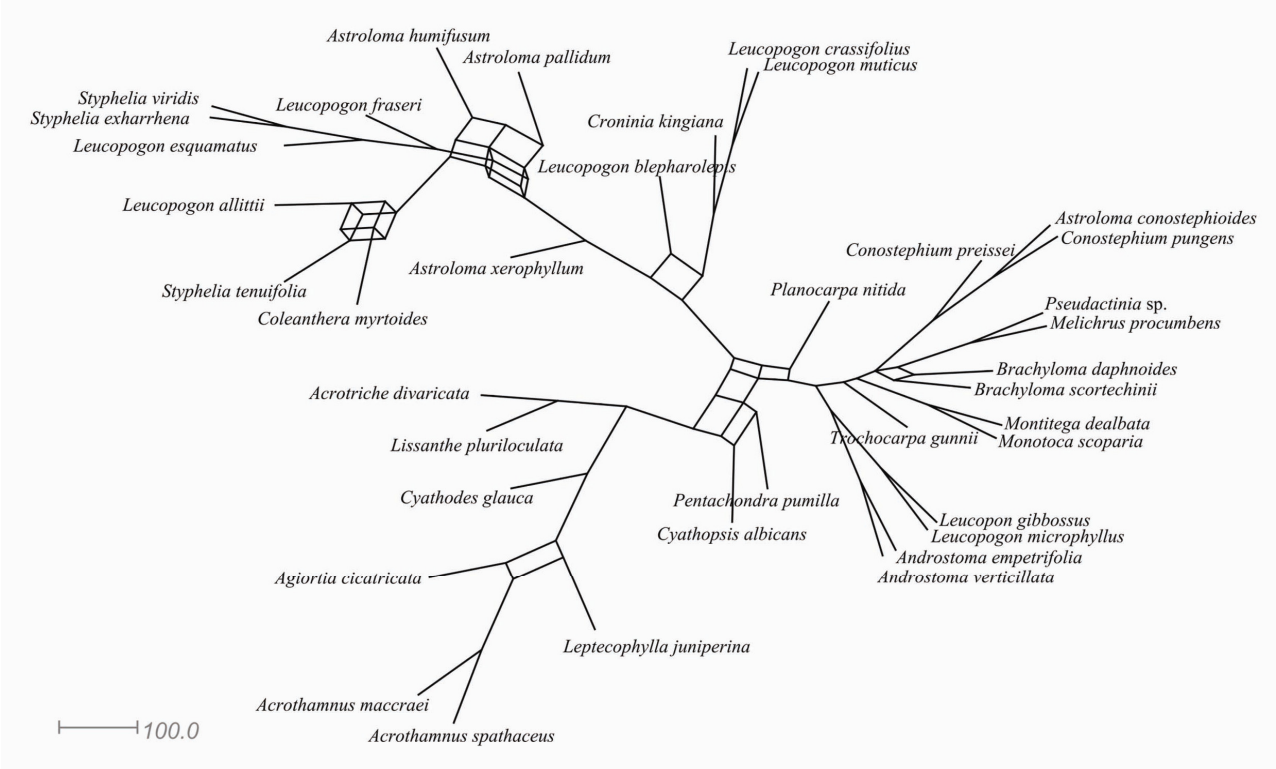


Figure 6-3 Consensus network displaying conflict within the Styphelieae, based on the 100 bootstrap trees from the RAxML five gene pruned large data set. All splits with > 15% bootstrap support are displayed. Splits are represented by sets of parallel edges, and the length of these edges is drawn proportional to the bootstrap support for that split.

Table 6-3 Support given to the relationships between tribes by supermatrix and supertree methods (support shown as % bootstrap for RAxML and MRP and posterior probability for MrBayes, 3 = three gene data set, 5 = five gene data set, P = Partitioned model, UP = Unpartitioned model)

	RAxML small data set				RAxML large data set				MrBayes small data set		MRP	
	3P	3UP	5P	5UP	3P	3UP	5P	5UP	3	5	3	5
(ES)	97	95	89	93	87	95	91	96	1	1	79	78
CES	45	47	44	47	44	49	37	53	.492	.471	28	19
RES	51	48	46	44	46	46	54	42	.501	.525	21	19
RCES	94	86	85	80	83	89	86	84	1	1	58	57
ORCES	72	87	83	76	80	86	89	69	.995	.988	51	41

Table 6-4 Support given to the position of *Agiortia* and *Cyathopsis* by supermatrix and supertree methods (support shown as % bootstrap for RAxML and MRP and posterior probability for MrBayes, 3 = three gene data set, 5 = five gene data set, P = Partitioned model, UP = Unpartitioned model, a = sister to *Leptecophylla*, b = grouped with *Leucopogon spathaceus* and *Acrothamnus maccraei*, c = sister to the Styphelieae, d = appears within the Styphelieae)

	RAxML small data set				RAxML pruned data set		MrBayes small data set		MRP	
	3P	3UP	5P	5UP	3	5	3	5	3	5
Agiortia cicatricatus	56 a	-	54 a	54 a	67 b	71 b	.671 a	.71 a	-	-
Cyathopsis albicans	-	-	51 c	-	-	55 c	.514 d	.544 d	-	-

6.4.3 Supertree Analyses

The three gene and five gene MRP analysis returned 7443 and 7440 equally parsimonious trees with scores of 179 and 191 on matrices with 136 and 143 characters respectively. No conflicts were found when we compared the supertrees using either three or five genes and either the standard MRP or the hierarchical bootstrap approach (Burleigh et al., 2006) (Fig. 6-4). Both the three and five gene standard MRP trees gave Richeeae basal compared to Cosmelieae. However, the hierarchal bootstrap approach gave a slightly more mixed view. Of the 100 trees in the five gene hierarchical bootstrap sample 19 gave PAOC | RES; and 19 gave PAOR | CES; and the remaining 62 mostly returned a three-way polytomy that did not resolve whether Cosmelieae or Richeeae were more basal. In the three gene hierarchical bootstrap sample 21 gave PAOC | RES; and 28 gave PAOR | CES; and the remaining 51 mostly returned a three-way polytomy that did not resolve whether Cosmelieae or Richeeae were more basal.

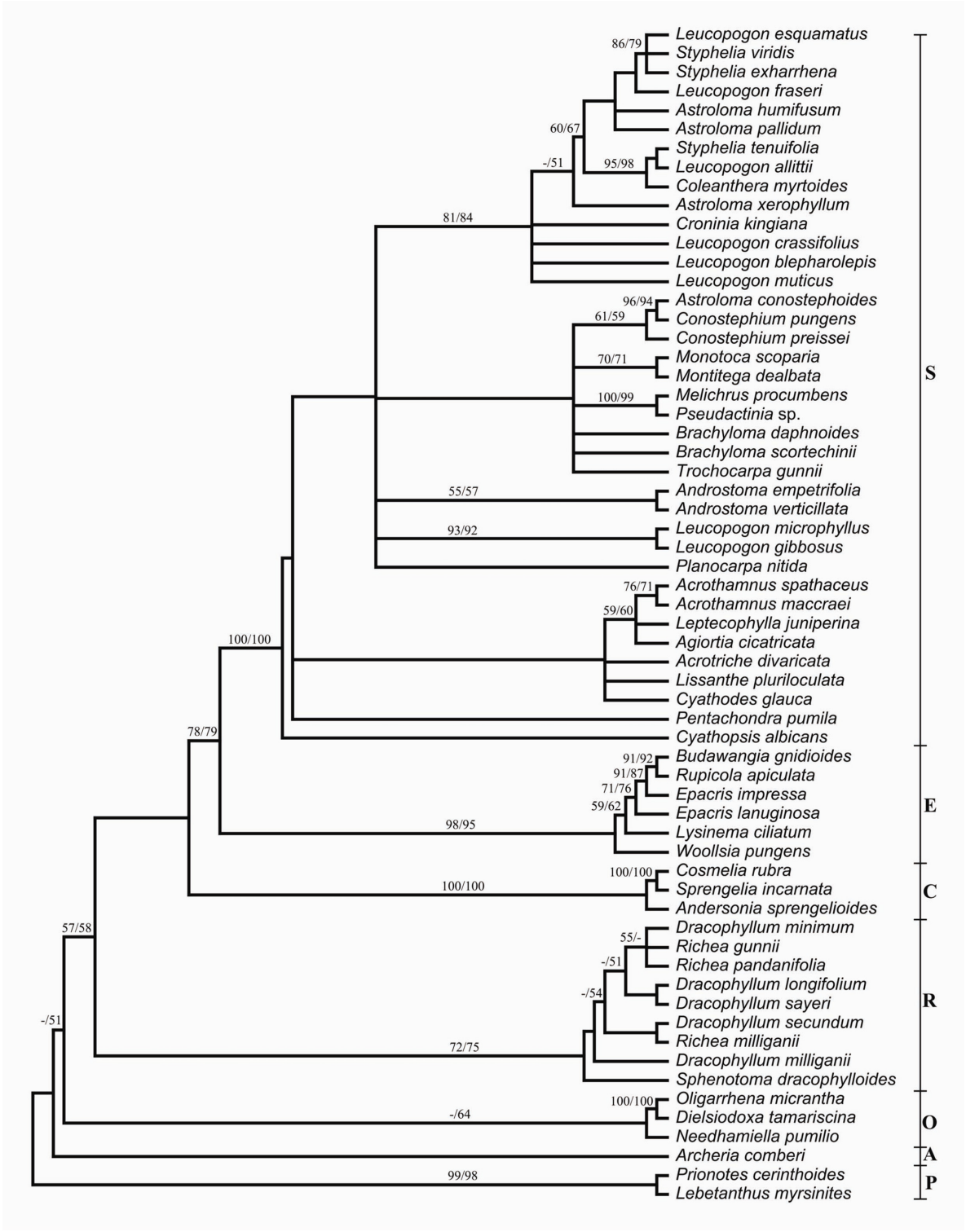


Figure 6-4 MRP supertree (based on five gene data set showing splits > 50%, annotated with hierarchical bootstrap values for five gene small data set / three gene small data set; position of tribes annotated: P = Prionoteae, A = Archerieae, O = Oligarrheneae, C = Cosmelieae, R = Richeeae, E = Epacrideae, S = Styphelieae).

6.4.4 Effects of taxon and character sampling on resolution

To facilitate the comparison of trees of different sizes, we pruned the bootstrap trees from each of the RAxML analyses on the large data sets (111 taxa) so that they only contained the same 62 taxa as found in the small data set. Table 6-5 compares the shared splits that have > 50% bootstrap support between data sets. Considering only splits with > 50% support, the highest bootstrap support was received by the five gene pruned data set (summed bootstrap of 4107 from 45 splits), followed by the five gene small data set (summed bootstrap of 3938 from 45 splits), the three gene pruned data set (summed bootstrap of 3876 from 42 splits), and the three gene small data set (summed bootstrap of 3796 from 42 splits). In all cases, the pruned data sets had more splits with greater support than the small data sets. Comparing the five gene to the three gene datasets gave more mixed results with the small data set resolving better with three genes in the Raxml analysis, but better with five genes in the MrBayes analysis. For MrBayes the three gene data resulted in a tree with 50 non-trivial splits (out of a possible 59) with greater than 0.50 posterior probability (average posterior probability of 0.906). The five gene data resulted in a tree with 49 non-trivial splits with greater than 50% posterior probability (average posterior probability of 0.922).

Table 6-5 Comparison of shared splits (> 50% bootstrap support) in RAxML hypotheses for five and three gene pruned and small data sets (top 4 comparisons), and comparison of shared splits (> 0.5 posterior probability) in MrBayes for five and three gene data sets.

Data set	# Splits with greater support	# Splits with equal support	# Splits shared
Comparison of five gene data sets			
5 gene, large pruned	21	13	41
5 gene, small	7		
Comparison of three gene data sets			
3 gene, large pruned	13	16	38
3 gene, small	9		
Comparison of large data sets - pruned			
5 gene, large pruned	16	17	41
3 gene, large pruned	8		
Comparison of small data sets (RAxML)			
5 gene, small	8	16	40
3 gene, small	16		
Comparison of small data sets (MrBayes)			
5 gene, small	15	25	48
3 gene, small	8		
Comparison of small data sets (MRP)			
5 gene, small	10	4	26
3 gene, small	12		

6.5 Discussion

Overall, the supermatrix and supertree approaches concurred in the tree topologies they returned. As expected, the supertree approaches were more conservative, providing less resolution at the genus level than either of their supermatrix counterparts. However, the similarity in tree topology between these philosophically different approaches provides confidence in the hypotheses returned; and suggests that the supermatrix hypotheses were free from systematic error due to model misspecifications caused by forcing all genes to have the same branch lengths (Kolaczowski and Thornton, 2004).

We found that support for the monophyly and position of the tribes and genus level relationships increased from the RAxML single gene hypotheses (refer supplementary 6-1 to 6-5) to the RAxML hypotheses based on the three and five gene data sets, regardless of the missing data. These tribal relationships also received strong posterior support in both the MrBayes three and five gene analyses. The MrBayes analyses provided noticeably greater support for many of the genus-level relationships, particularly in the Styphelieae. Such discrepancies in the level of support between nonparametric bootstrap values and Bayesian posterior probabilities are a widely recognised phenomenon. Although Douady et al. (2010) have confirmed that posterior probabilities tend to be less conservative than bootstrap proportions, Wilcox et al. (2002) found that higher Bayesian support values for relationships between dwarf boas represented better estimates of phylogenetic accuracy than the nonparametric bootstrap values. Lemmon et al. (2009) found that in both ML and Bayesian frameworks among-site rate variation could interact with missing data and result in erroneous estimates of topology and branch lengths. They found that priors on branch lengths and rate heterogeneity could exacerbate misleading bipartition posterior probabilities in Bayesian analyses. It is suggested that where bipartitions are strongly supported by non-missing sites they are likely to remain strongly supported even if missing sites are included; but that where bipartitions are weakly supported they may become strongly supported with the addition of just a few missing sites (Lemmon et al., 2009). In our interpretation of phylogenetic hypotheses of the Styphelioideae, we mitigated against such errors by placing confidence in areas of strong agreement between ML and Bayesian analyses. In addition, we looked for consistency in the hypotheses from supermatrix and supertree methods.

We found that the monophyly of the tribes was consistent between the supermatrix and supertree approaches and consistent with previous studies (Kron et al., 1999a; Crayn and Quinn, 2000; Quinn et al., 2003; Wagstaff et al., 2010). With the exception of Richeeae and Cosmelieae, the position of the tribes was well-supported in the supermatrix analyses and occupied the same positions but with less support in the supertree analyses. However there was no Bayesian support for Richeeae as one node basal to Cosmelieae (0.525 posterior probability in five gene data set and 0.501 in three gene data set) and extremely weak support for Cosmelieae as one node basal to Richeeae (57% bootstrap support in hierarchical supertree in five gene data set and 58% in three gene data set; RAxML returned variable results but all with low support). In past analyses, Wagstaff et al. (2010) received good support for Richeeae having diverged one node basal to Cosmelieae in their combined *rbc*L and *mat*K Bayesian analyses (0.99 posterior probability) and parsimony analyses (95% bootstrap support). The relationship was unresolved in their separate *rbc*L analyses, and Richeeae occurred in a polytomy with Epacrideae and Cosmelieae in the *mat*K analyses. Likewise, our *mat*K RAxML analyses gave only weak support to the Richeeae and Cosmelieae grouping, while our RAxML *rbc*L analyses had Cosmelieae outside of Richeeae, again, weakly supported. Other phylogenetic hypotheses had the relationship as unresolved or showed low bootstrap support for Richeeae as one step basal to Cosmelieae, or Cosmelieae as one step basal to Richeeae (Crayn et al., 1996; Crayn et al., 1998; Kron et al., 1999a; Crayn and Quinn, 2000). Although there has been some support for Richeeae having diverged basal to Cosmelieae, there is enough discrepancy across all analyses to suggest that the resolution of the relationship would benefit from sequencing additional markers.

Within the tribes the well-supported occurrence of *Dielsiodoxa* in the Oligarrhenaeae as sister to *Oligarrhena* rather than within the Styphelieae (supported by Mr Bayes, RAxML and supertree analyses; and also in *mat*K and *atp*β-*rbc*L intergenic spacer single gene RAxML analyses) supported the findings of Quinn et al. (2003) (where this sample was labelled by its old name *Monotoca tamariscina*) and Albrecht et al. (2010). Within Richeeae, our results were consistent with those of Wagstaff et al. (2010) - *Sphenotoma* is sister to the paraphyletic *Richea* and *Dracophyllum*. Genus arrangement within the Cosmelieae was also consistent with Wagstaff et al. (2010) - *Andersonia* is sister to *Cosmelia* and *Sprengelia*. Similar to Crayn and Quinn (2000), we found *Epacris* Cav. and *Rupicola* to be paraphyletic and

Woollsia to be sister to the rest of the Epacrideae.

In general, further scrutiny needs to be applied to genus-level relationships in the Styphelieae. Regardless of this, we found support for the monophyly of *Acrotriche* R.Br., *Leptecophylla* C.M.Weiller, *Pentachondra* R.Br., and *Planocarpa* C.M.Weiller, supporting previous work by Quinn et al. (2003) (Supplementary 6-1 to 6-5). In addition, we received support for the monophyly of *Acrothamnus* and *Monotoca*. Several other groups resolved by Quinn et al. (2003) received improved support in our RAxML large data set analyses: the *Acrotriche* clade (*Acrotriche*, *Lissanthe* R.Br.), *Conostephium* clade (*Astroloma conostephioides* (Sond.) F.Muell. ex Benth., *A. pinifolium* (R.Br.) Benth., *Conostephium* Benth.), *Melichrus* clade (*Melichrus*, '*Pseudactinia*'), and *Monotoca* clade (*Monotoca*, *Montitega*). Overall, our work provides a greater level of confidence in the phylogenetic accuracy of these groups.

We established that (in RAxML) choices in gene number, partitioning or no partitioning, and whether or not sites were invariant made very little difference to the tree topology. We found that inclusion of a larger number of incomplete taxa in the RAxML supermatrix improved the level of support received even if some of the taxa were later removed. That is, we got better bootstrap support when phylogenetic hypotheses were based on our large data set with either 58% missing data (three gene) or 66% missing data (five gene) and then pruned to the size of our small and more complete data set, either 37% missing data (three gene) or 49% missing data (five gene), than when we used the small data set from the beginning. These results support the use of incomplete taxa in forming phylogenetic hypotheses using a ML supermatrix approach for the Styphelioideae.

Wiens (2006) established that taxa that were 50% complete were just as beneficial as taxa that were 100% complete. In a study of hylid frog phylogeny, Wiens et al. (2005) included taxa that were < 10% complete, and still found that the resulting trees placed all taxa in the expected higher-level clades. Like Wiens et al. (2005), we had some taxa that were < 10% complete in our large data set. We found that incomplete taxa did not adversely affect tree topology, but improved resolution at the genus-level. Furthermore, using consensus networks to examine marginally supported (bootstrap > 10%) links between taxa, we were able to confirm that our trees were not affected by missing data and were free from "wildcard" taxa described by Malia et al. (2003) - taxa that switch position in the tree causing low bootstrap support. It is possible that the data set of the Styphelioideae included enough taxa with

overlapping site completeness to mimic the "scaffold" technique outlined by Wiens et al. (2005).

While our approach was based on data availability rather than the more strategic approach of Wiens et al. (2005), it still resulted in a form of "scaffold". Our supermatrix had at least one taxon represented by data for three genes in each of the 7 tribes of the Styphelioideae. In our most incomplete data set (large data set) we had no taxa represented by five genes, 4% taxa represented by 4 genes, 27% taxa represented by three genes, 54% taxa represented by 2 genes, and 15% taxa represented by only the *atpB-rbcL* intergenic spacer. However, the spacer had the most widespread sampling across the subfamily, providing an overlap of data with other closely-related taxa which, in turn, were represented by more gene samples. This may assist in phylogenetic estimation as the spacer *holds* the data set together. The spacer is known to improve resolution (from genes such as *rbcL*), among the major lineages and at lower hierarchical levels, including in the problematic Styphelieae (Quinn et al., 2003). However, Quinn et al. (2003) found that the additional structure provided by the spacer was not well-supported. Thus, it is likely that the increased support in our phylogenetic hypotheses may be due to the different methods used, particularly the supermatrix approach that has the potential to provide support that is not always apparent when fewer genes are used.

Similar to Wiens et al. (2005) we had a combination of slow-evolving sites from *rbcL* and *matK*, and more rapidly evolving sites from spacer, 18S and ITS. In contrast to Wiens et al. (2005), our data set had a denser sampling of taxa, the majority of taxa were represented by > 1 gene, and we had fewer complete taxa. Our work suggests that a "scaffold" may take an *ad hoc* form in addition to the more structured approach indicated by Wiens et al. (2005). This opens up the possibility for a more widely used "scaffold" approach. In general, where sequencing within a related-group has included a mix of slow evolving sites to resolve higher level relationships and more rapidly evolving sites to resolve relationships closer to the tree-tips, it is possible to unite them to answer broader phylogenetic questions about the group. The "scaffold" approach is particularly useful where the amount and type of character sampling necessary to achieve adequate resolution is variable between tribes. In our case, the species-rich Styphelieae is likely to be in need of more taxa and more gene sampling than the rest of the Styphelioideae to resolve its genus-level relationships.

The multi-gene approach has led to a more complete, more resolved and more robust genus-

level phylogenetic hypothesis of the Styphelioideae. We found that by comparing the supermatrix and supertree hypotheses, the origin of any conflicts were clearer, such as whether they were from the data used, or assumptions made. We found that using a data set of variably complete taxa provided results that concurred with past phylogenetic work in the Styphelioideae and in addition provided greater resolution and support for tribe and genus-level splits. In the past, concerns around the effects of missing data have meant that incomplete taxa were often excluded from analyses. While we recommend a cautious interpretation of phylogenetic estimates where incomplete taxa have been included, we support the notion that where different approaches concur we may have confidence in the phylogenies returned. For the Styphelioideae, the morphologically diverse *Astroloma-Styphelia* clade of Styphelieae remains the main problem area for resolving the genus-level relationships of the subfamily.

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6.6 References

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6.7 Appendix

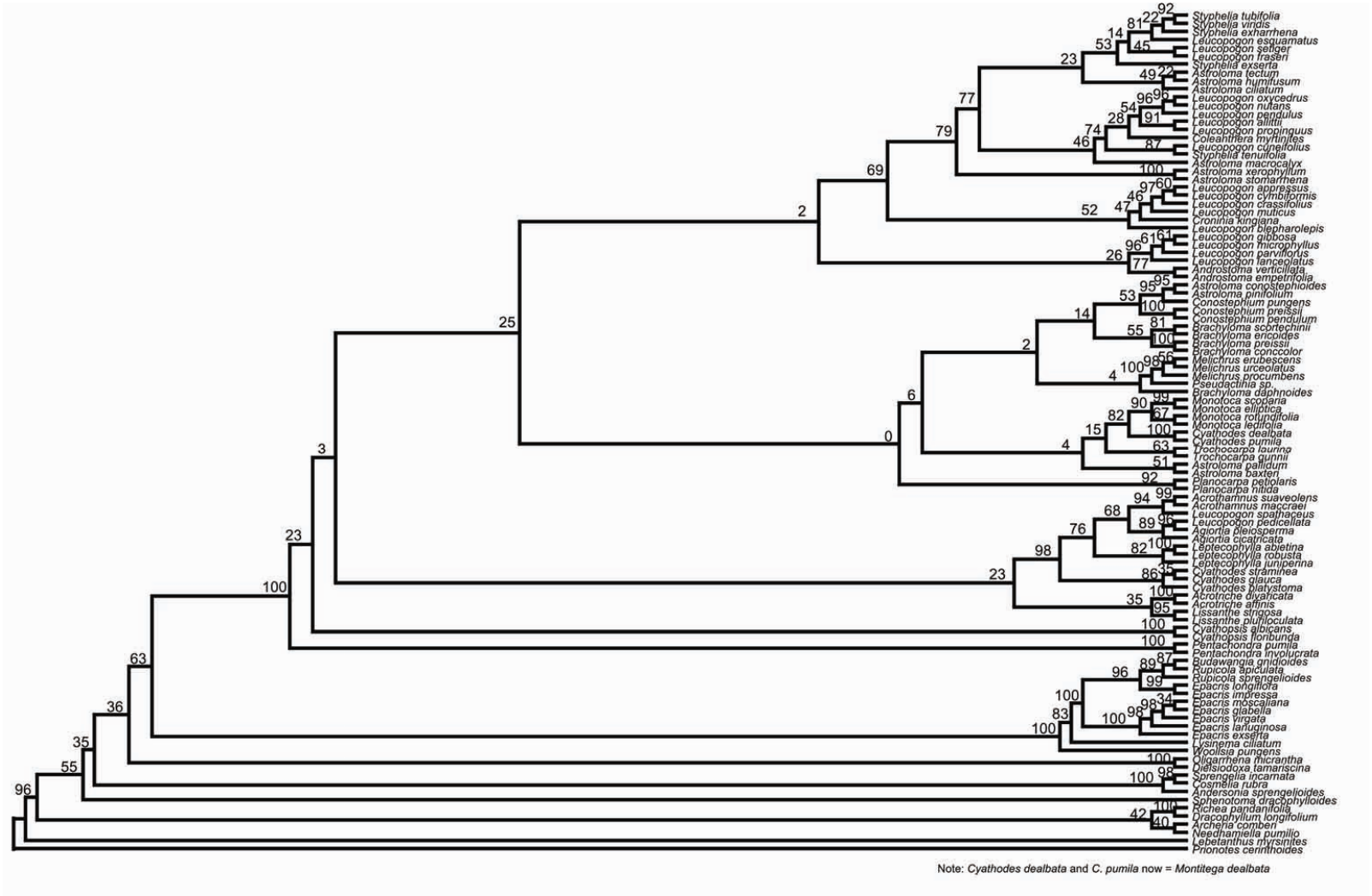
Appendix 6-1 GenBank accession numbers for sequences used in analyses (bold type = small data set)

Species	Spacer	RbcL	MatK	18S	ITS
Acrothamnus colensoi (Hook.f.) Quinn [was *Styphelia*]	*	L12610.2	*	*	*
Acrothamnus maccraei (F.Muell.) Quinn	**AF208778.1**	*	**AY372644.1**	*	*
Acrothamnus spathaceus (Pedley) Quinn (was *Leucopogon*)	**AY372580.1**	*	**AY372656.1**	*	*
Acrothamnus suaveolens (Hook.f.) Quinn	AY372589.1	*	AY372668.1	*	*
Acrotriche affinis DC.	AY372541.1	*	*	*	*
Acrotriche divaricata R.Br.	**AF155855.2**	**U80432.1**	**AY005094.1**	*	*
Agiortia cicatricata (J.M.Powell) Quinn	AY372572.1	*	AY372632.1	*	*
Agiortia cicatricata (J.M.Powell) Quinn [was ***Leucopogon cicatricatus*]**	**AY372572**	*	**AY372632.1**	*	*
Agiortia pedicellata (C.T.White) Quinn [was *Leucopogon pedicellatus*]	AY372577.1	*	AY372649.1	*	*
Agiortia pleiosperma (F.Muell.) Quinn	AY372578.1	*	*	*	*
Andersonia sprengelioides R.Br.	**AF155843.2**	**U79742.1**	**AF015631.1**	*	*
Androstoma empetrifolia Hook.f.	**AY372540.1**	*	**AY372597.1**	*	*
Androstoma verticillata (Hook.f.) Quinn [was ***Leucopogon milliganii*]**	**AY372575.1**	*	**AY372645.1**	*	*
Archeria comberi Melville	**AF155840.2**	**U79741.1**	**AF015632.1**	*	*
Astroloma baxteri A.Cunn. ex DC.	AY372543.1	*	AY372598.1	*	*
Astroloma ciliatum (Lindl.) Druce	AF208748.1	*	AY372599.1	*	*
Astroloma conostephioides (Sond.) F.Muell. ex Benth.	**AY372544.1**	*	**AY372600.1**	*	*
Astroloma humifusum (Cav.) R.Br.	**AF155866.2**	**U80433.1**	**AY372602.1**	*	**JF437579.1**
Astroloma macrocalyx Sond.	AY372547.1	*	AY372603.1	*	*
Astroloma pallidum R.Br.	**AY372548.1**	*	**AY372604.1**	*	*
Astroloma pinifolium (R.Br.) Benth.	AY372549.1	*	AY372605.1	*	*
Astroloma stomarrhena Sond.	AY372550.1	*	*	*	JF437569.1
Astroloma tectum R.Br.	AY372551.1	*	AY372606.1	*	JF437576.1
Astroloma xerophyllum (DC.) Sond.	**AY372554.1**	*	**AY372607.1**	*	**JF437570.1**
Brachyloma concolor (F.Muell.) Benth.	JF437581.1	*	*	*	*
Brachyloma daphnoides (Sm.) Benth.	**AF155859.2**	**U80428.1**	**AF015633.1**	*	*
Brachyloma ericoides (Schltdl.) Sond.	JF437582.1	*	*	*	*
Brachyloma preissii Sond.	AY372555.1	*	AY372610.1	*	*
Brachyloma scortechinii F.Muell.	**AF208749.1**	*	**AY372611.1**	*	*
Budawangia gnidioides (Summerh.) I. Telford	**AF155852.2**	**AF156095.1**	*	*	*
Coleanthera myrtoides Stschegl.	**AY372556.1**	*	**AY372612.1**	*	**JF437574.1**
Conostephium pendulum Benth.	**JF437583.1**	*	**AY372613.1**	*	*
Conostephium preissii Sond.	**AY372557.1**	*	**JF437592.1**	*	*
Conostephium pungens Keighery	**JF437584.1**	*	*	*	*
Cosmelia rubra R.Br.	**AF155842.2**	**U80420**	**AF015634 .1**	**AF419801.1**	*
Croninia kingiana (F.Muell.) J.M.Powell	**AF208750.1**	*	**AY372614.1**	*	**JF437568.1**
Cyathodes glauca Labill.	**AF155858.1**	**U80434.1**	**AY005095.1**	*	*
Cyathodes platystoma C.M.Weiller	AY372560.1	*	AY372616.1	*	*
Cyathodes straminea R.Br.	AF208752.1	*	AY372617.1	*	*
Cyathopsis albicans (Brongn. & Gris) Quinn	**AY636039.1**	*	**AY636039.1**	*	*
Cyathopsis floribunda Brongn. & Gris	AY636040.1	*	AY636040.1	*	*

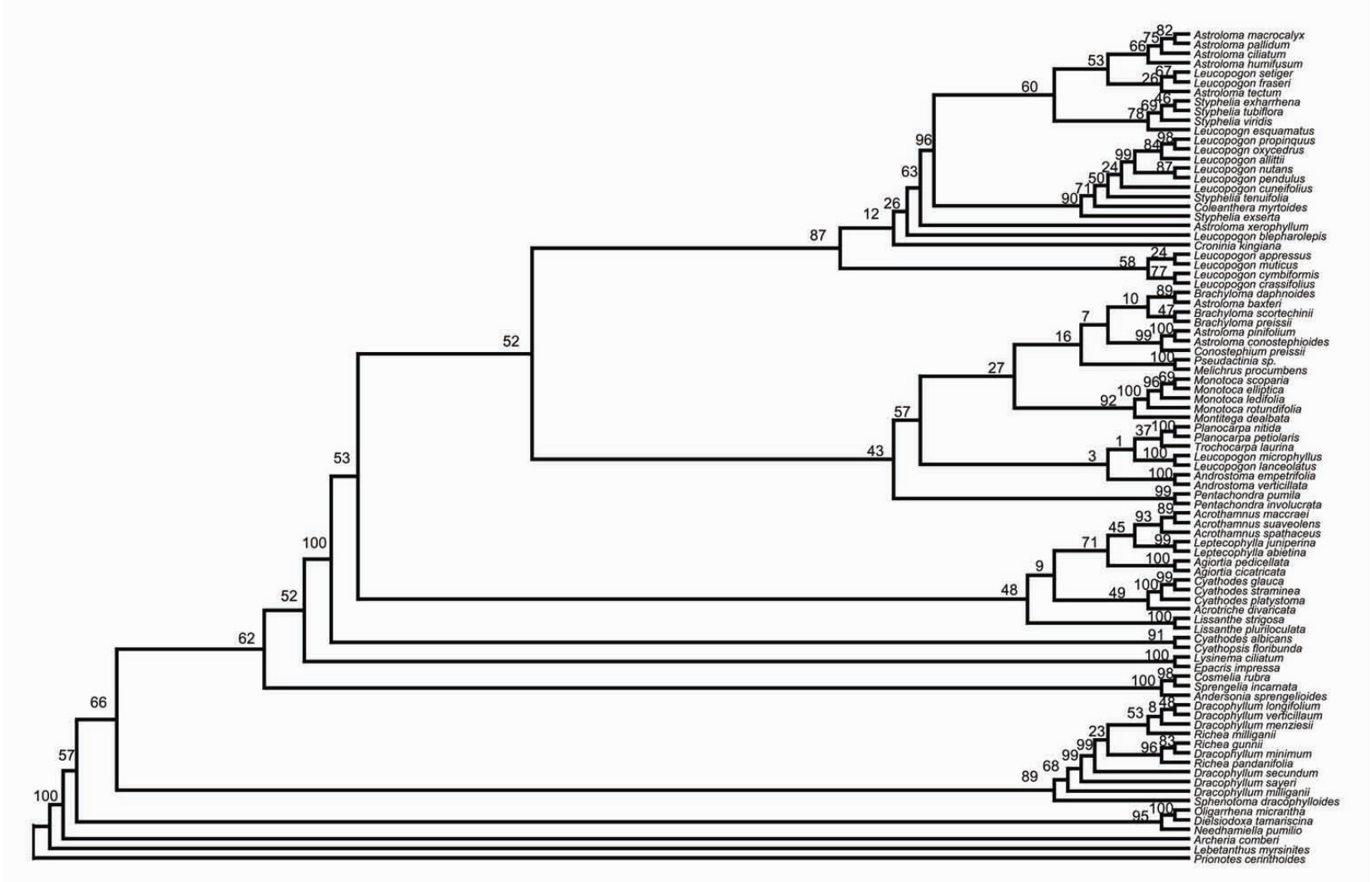
Species	Spacer	RbcL	MatK	18S	ITS
Dielsiodoxa tamariscina (F.Muell.) Albr. [was ***Monotoca***]	**AY372586.1**	*	**AY372662.1**	*	*
Dracophyllum longifolium R.Br. ex Roem. & Schult.	*	L12614.2	AF015635.1	*	*
Dracophyllum menziesii	AF155845	GQ392910.1	GQ392962.1	*	*
Dracophyllum milliganii Hook.f.	*	**GQ392911.1**	**GQ392963.1**	*	*
Dracophyllum minimum F.Muell	*	**GQ392912.1**	**GQ392964.1**	*	*
Dracophyllum sayeri F.Muell.	*	**GQ392921.1**	**GQ392973.1**	*	*
Dracophyllum secundum R.Br.	*	**GQ392924.1**	**GQ392976.1**	*	*
Dracophyllum verticillatum	*	GQ392930.1	GQ392982.1	*	*
Epacris exserta R.Br.	JF437585.1	*	*	*	*
Epacris glabella Jarman	JF437586.1	*	*	*	*
Epacris impressa Labill.	**AF155849.2**	*	*	*	*
Epacris lanuginosa Labill.	**AF155850.2**	**U80426.1**	*	*	*
Epacris longiflora Cav.	JF437587.1	*	*	*	*
Epacris moscaliana Crowden	JF437588.1	*	*	*	*
Epacris virgata Hook.f.	JF437589.1	*	*	*	*
Lebetanthus myrsinites	**AF155839.2**	**U81797.1**	**AF539983.1**	*	*
Leptecophylla abietina (Labill.) C.M.Weiller	AY372561.1	*	AY372618	*	*
Leptecophylla juniperina (R.Br.) C.M.Weiller	**AY372566.1**	*	**AY372624**	EF635454.1	*
Leptecophylla robusta (Hook.f.) C.M.Weiller	AY372568.1	*	*	*	*
Leucopogon allittii F.Muell.	**AF208753.1**	*	**AY372627**	*	**JF437571.1**
Leucopogon appressus R.Br.	AF208756.1	*	AY372630.1	*	*
Leucopogon blepharolepis (F.Muell.) Benth.	**AY372571.1**	*	**AY372631**	*	*
Leucopogon crassifolius Sond.	**AF208764.1**		**AY372634.1**		
Leucopogon cuneifolius Stschegl.	AF208765.1	*	AY372635	*	JF437573.1
Leucopogon cymbiformis A.Cunn. ex DC.	AF208766.1	*	AY372636	*	*
Leucopogon esquamatus R.Br.	**AF208769.1**	*	**AY372638**	*	**JF437577.1**
Leucopogon fraseri DC.	**AY005084.1**	**L12620.2**	**AY005096.1**	*	*
Leucopogon fraseri DC. NSW	AF208771.1	*	AY372639.1	*	*
Leucopogon gibbosus Stschegl.	**AF155863.2**	**U79739**	*	*	*
Leucopogon lanceolatus (Sm.) R.Br.	AF208776.1	*	AY372642.1	*	*
Leucopogon microphyllus (Cav.) R.Br.	**AF155862.2**	**U79740.1**	**AY005097.1**	*	*
Leucopogon muticus R.Br.	**AF155864.2**	**U80429.1**	**AF015638.1**	*	*
Leucopogon nutans E.Pritzel	AF208780.1	*	AY372647.1	*	*
Leucopogon oxycedrus Behr & F.Muell. ex Sond.	AF208782.1	*	AY372648.1	*	*
Leucopogon parviflorus (Andrews) Lindl.	AF208783.1	*	*	EF635456	*
Leucopogon pendulus R.Br.	AF208784.1	*	AY372650.1	*	*
Leucopogon propinquus R.Br.	AF208788.1	*	AY372654.1	*	*
Leucopogon setiger R.Br.	AF208790.1	*	AY372655.1	*	JF437572.1
Lissanthe pluriloculata (F.Muell.) J.M.Powell, Crayn & E.A.Br. [was ***Leucopogon pluriloculatus***]	**AF208786.1**	*	**AY372652.1**	*	*
Lissanthe strigosa (Sm.) R.Br.	AF208794.1	*	AY372658.1	*	*
Lysinema ciliatum R.Br.	**AF155848.2**	**U80424**	**AF015639.1**	*	*
Melichrus erubescens A.Cunn. ex DC.	JF437590.1	*	*	*	*
Melichrus procumbens (Cav.) Druce	**AF155856**	**U80430**	**AY005098.1**	*	*
Melichrus urceolatus R.Br.	AY372595.1	*	*	*	*
Monotoca elliptica (Sm.) R.Br.	AY005085.1	*	AY005099.1	*	*
Monotoca ledifolia A.Cunn ex DC.	AY372584.1	*	AY372660.1	*	*
Monotoca rotundifolia J.H.Willis	AY372585.1	*	AY372661.1	*	*
Monotoca scoparia (Sm.) R.Br.	**AF155857.2**	**U80431**	**AF015640.1**	*	*

Species	Spacer	RbcL	MatK	18S	ITS
Montitega dealbata (R.Br.) C.M.Weiller [was ***Cyathodes*****]**	**AY372559.1**	*	**AY372615.1**	*	*
Montitega dealbata (R.Br.) C.M.Weiller [was *Cyathodes pumila*]	AY372558.1	*	*	*	*
Needhamiella pumilio (R.Br.) L.Watson	**AF155853.2**	**U80422**	**AF539984.1**	*	*
Oligarrhena micrantha R.Br.	**AF155854.2**	**U80423**	**AF539985.1**	*	*
Pentachondra involucrata R.Br.	AY005087.1	*	AY005101.1	*	*
Pentachondra pumila (J.R.Forst. & G.Forst.) R.Br.	**AF155860.2**	**L12621.2**	**AF015641.1**	**AF419805**	*
Planocarpa nitida (Jarman) C.M.Weiller	**AY372593.1**	*	**AY372663.1**	*	*
Planocarpa petiolaris (DC.) C.M.Weiller	AY372594.1	*	AY372664.1	*	*
Prionotes cerinthoides (Labill.) R.Br.	**AF155838.2**	**U79743**	**AF015642.1**	**AF419806**	*
Pseudactinia sp.	**AY372596.1**	*	**AY372665.1**	*	**JF437567.1**
Richea gunnii Hook.f.	*	**GQ392934.1**	**GQ392986.1**	*	*
Richea milliganii Hook.f. F.Muell.	*	**GQ392935.1**	**AY372669**	*	*
Richea pandanifolia Hook.f.	**AF155844.2**	**U80418.1**	**AF539986.1**	*	*
Rupicola apiculata (A.Cunn.) I.Telford	JF437591.1	*	*	*	*
Rupicola sprengelioides Maiden & Betche	**AF155851.2**	*	*	*	*
Sphenotoma dracophylloides Sond.	**AF155846.2**	**U80419.1**	**AF015644**	*	*
Sprengelia incarnata Sm.	**AF155841.2**	**U80421.1**	**AF015645**	*	*
Styphelia exarrhena (F.Muell.) F.Muell.	**AY372587.1**	*	**AY372666.1**	*	**JF437575.1**
Styphelia exserta (F.Muell.) Sleumer	AY372588.1	*	AY372667.1	*	*
Styphelia suaveolens (Hook.f.) Warb.	AY372589.1	*	AY372668.1	*	*
Styphelia tenuifolia Lindl.	**AY372590.1**	*	**AY372669.1**	*	**JF437580.1**
Styphelia tubiflora Sm.	AY372591.1	*	AY372670.1	*	JF437578.1
Styphelia viridis Andrews	**AF155865.2**	**U81798.1**	**AY005105.1**	*	*
Trochocarpa gunnii (Hook.f.) Benth.	**AF155861.2**	**U81799.1**	*	*	*
Trochocarpa laurina (Rudge) R.Br.	AY005092.1	*	AY005106.1	*	*
Woollsia pungens (Cav.) F.Muell.	**AF155847.2**	**U80425.1**	*	*	*

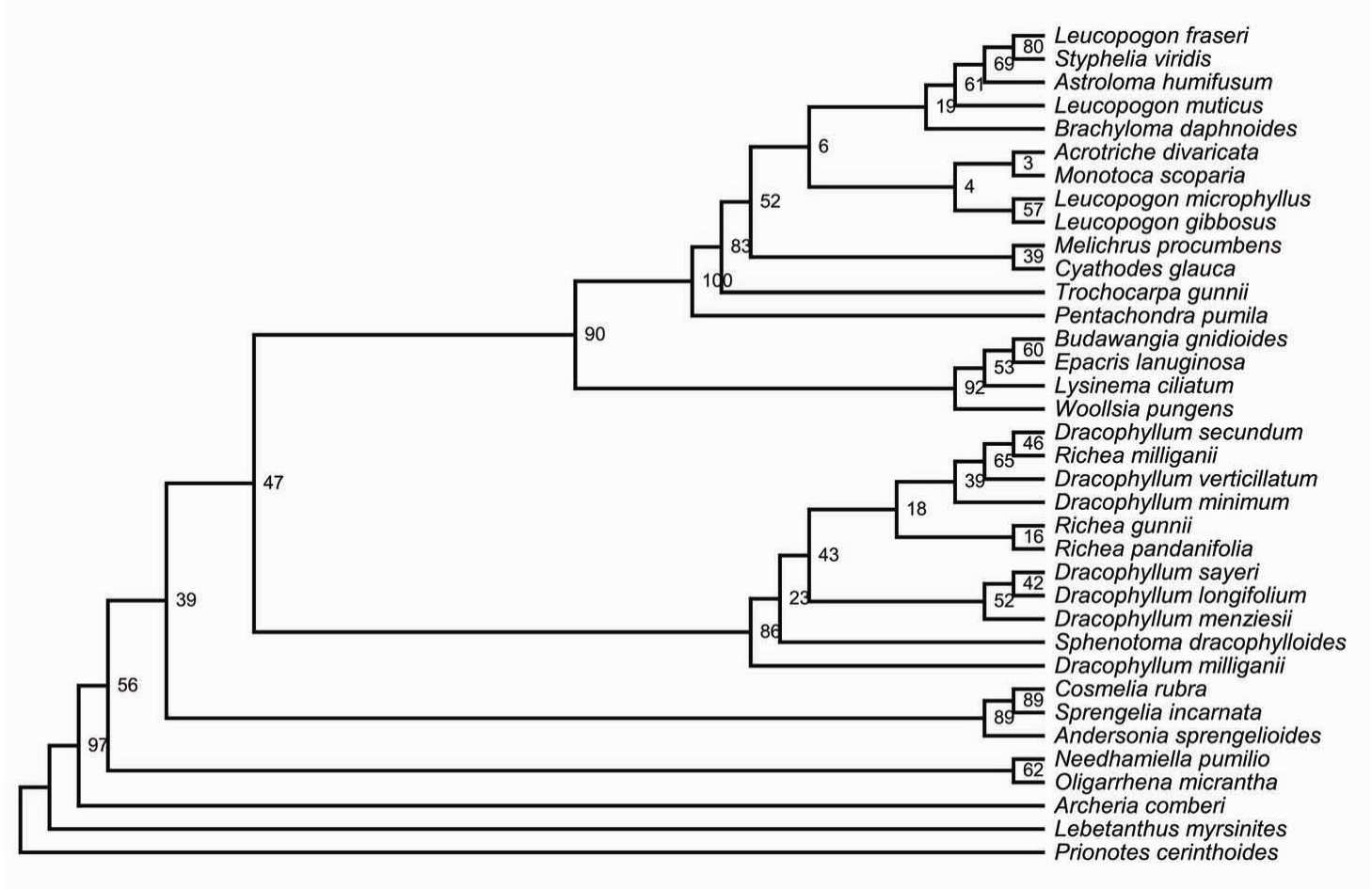
6.8 Supplementary information



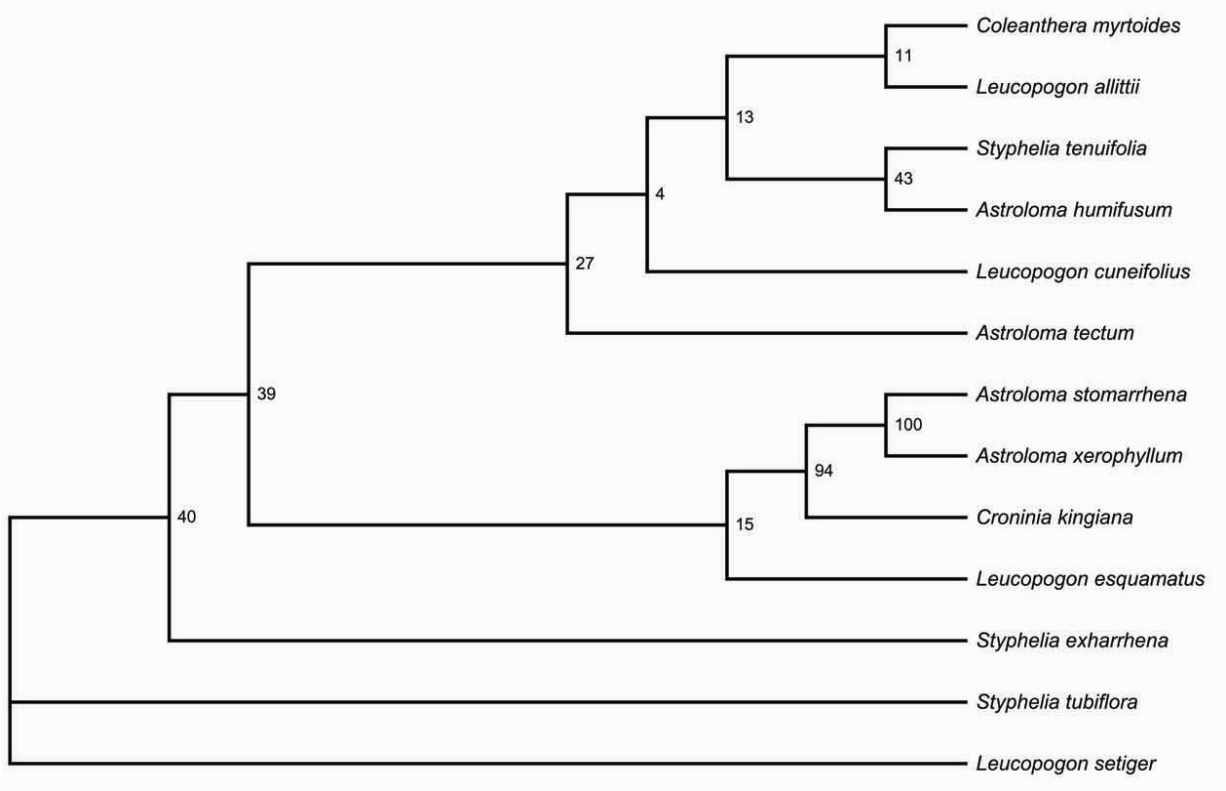
Supplementary 6-1 RAxML tree - *atp*β-*rbc*L intergenic spacer



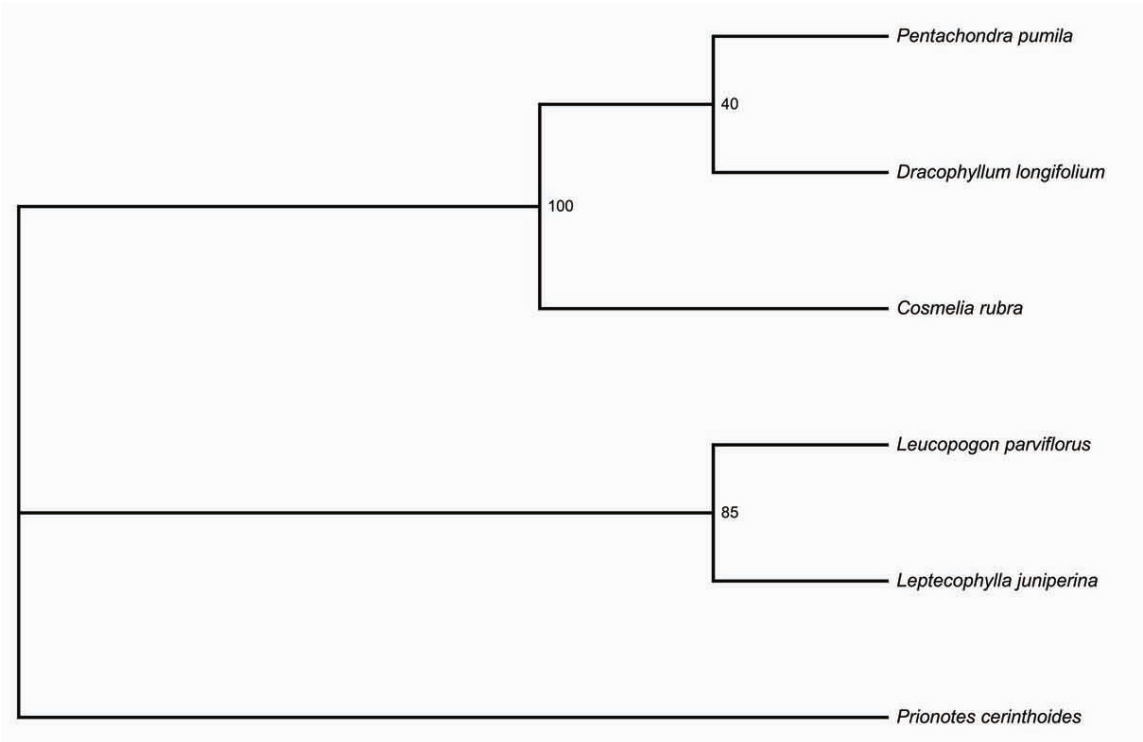
Supplementary 6-2 RAxML tree - *mat*K



Supplementary 6-3 RAxML tree - *rbc*L



Supplementary 6-4 RAxML tree - ITS



Supplementary 6-5 RAxML tree – 18S

Chapter 7 Revealing the evolution of epacrid pollination systems: Inferring syndromes from floral features and a known subset of pollinators

In preparation for journal submission: **Karen A. Johnson** (XXXX) Revealing the evolution of epacrid (Styphelioideae, Ericaceae) pollination systems: Inferring potential pollination systems for the speciose subfamily Styphelioideae (Ericaceae)

7.1 Abstract

Premise for the study: Where the same floral trait(s) have arisen independently in separate parts of the evolutionary tree, convergent evolution is indicated. Selection pressures exerted by pollinator(s) may play a part in this evolution. Here I explore the evolution of pollination systems of the Styphelioideae. I customise pollination syndromes from known plant and pollinator relationships and use them to infer pollination systems where there are gaps in knowledge.

Methods: Using non-metric multidimensional scaling and pairwise analyses I explore the relationships between floral features and pollinator profiles. Where pollinator groups are significantly related to floral traits, I use the Random Forests classification technique to obtain a set of floral attributes that are predictive of the relationship. I use these customised pollination syndromes to assign pollinator groups where they are unknown, and map them onto a phylogeny.

Key results: Floral trait profiles were significantly related to bird, bee, and fly visitors. Fly and bee pollination are widely supported by epacrid flowers, while red flowers and long floral tubes are the most important predictors for those visited by birds. Insect pollination is widespread across the evolutionary tree, while bird pollination appears in 16 separate locations, where it generally coincides with the occurrence of red flowers.

Conclusions: Customising pollination syndromes from known plant and pollinator relationships allows the accuracy of predictions to be gauged. Inferring pollination syndromes has led to a more complete hypothesis of the evolution of epacrid pollination systems.

7.2 Introduction

Where the same floral trait(s) occur independently in separate parts of the evolutionary tree, convergent evolution is indicated. Selection pressures exerted by pollinator(s) may play a part in this evolution. The recent development of a phylogenetic hypothesis for the Styphelioideae (Chapter 6), formerly family Epacridaceae, provides an opportunity to decipher an evolutionary history of floral traits and pollination systems within this subfamily. Known for its species richness (comprising 38 recognised genera and approx. 550 species) and floral diversity (Stephens, 2004) it provides an ideal system for determining the relationships between flower characters and pollinators. Although all species in the Styphelioideae have actinomorphic flowers there is much variety, from small white flowers in *Leucopogon* to large red tubular flowers in *Prionotes*; from *Richea* species that abscise their corollas to present a brush-like flower to the small green, hidden flowers of *Acrotriche* (Fig. 7-1). The prominence of the Styphelioideae, particularly in the Australian landscape, has led to a rich history of pollinator observations (Fletcher, 1977; Paton and Ford, 1977; Ford et al., 1979; Green and Osborne, 1994; Corbett, 1995; Keighery, 1996; Higham and McQuillan, 2000; Hingston and McQuillan, 2000; Houston, 2000; Houston and Ladd, 2002; Celebrezze and Paton, 2004; Johnson et al., 2010; Johnson et al., 2011; Schneemilch et al., 2011). Currently there is empirical information on the potential pollination systems for about 15% of the epacrid species across six of the seven tribes. However, in order to more fully explore the evolution of pollination systems within the subfamily it is useful to infer pollination systems where they are currently unknown.

Pollination syndromes have been used to both infer the pollinators of plant species in the absence of observations and to provide a mechanistic explanation of floral diversity (Fletcher, 1977; Fenster et al., 2004; Perez et al., 2006; Whittall and Hodges, 2007). However, they have been found to be unreliable predictors of major pollinators in some plant communities (Hingston and McQuillan, 2000; Ollerton et al., 2009). Furthermore, regional variability may make the applicability of Northern Hemisphere syndromes to Oceania inappropriate (Newstrom and Robertson, 2005). Applicability also differs across plant families, with Fabaceae, Apocynaceae and Asteraceae conforming better than other families (Ollerton et al. 2009). It is unlikely that a universally-applicable set of pollination syndromes will be discovered (Ollerton et al. 2009).

Traditional pollination syndromes seem most likely to be evident among plant species that rely on a guild of specialised pollinators (Goldblatt and Manning, 2006; Pauw, 2006), for instance, the rodent pollination syndrome (Johnson et al., 2001; Kleizen et al., 2008) and bird pollination syndrome (Armesto et al., 1996; Hargreaves et al., 2004; Botes et al., 2009). In contrast plants that invite a wide range of pollinators are unlikely to possess the traditional pollination syndromes (Manning and Goldblatt, 2005; Shuttleworth and Johnson, 2008). However, it is also possible that floral phenotypes may evolve under selection pressures exerted by various functional groups of pollinators (Martén-Rodríguez et al., 2009). Finally, the selection pressures exerted on floral parts by non-pollinators add a further level of complexity (Castellanos et al., 2004; Strauss and Whittall, 2006).

In response to such difficulties, researchers have tailored the syndrome concept to specific geographic areas and plant taxa (Thomson et al., 2000; Andersson et al., 2002; Kay and Schemske, 2003; Ollerton et al., 2003; Castellanos et al., 2006; Goldblatt and Manning, 2006; Buchanan, 2007). In speciose groups (such as the Styphelioideae) where a significant number of plant-pollinator relationships remain unknown, any known relationships can be examined for repeated patterns which may then be used to infer pollination mechanisms for additional species (Goldblatt and Manning, 2006). For instance, using pollination observations for 375 species of sub-Saharan Iridaceae based on repeated patterns of floral traits documented in the literature, Goldblatt and Manning (2006) inferred pollination systems for a further 610 species in the African Iridaceae.

The Styphelioideae consists of woody plants ranging from small prostrate shrubs to temperate rainforest emergents. Their range extends across SE Asia, Oceania, New Zealand, New Caledonia and Tierra del Fuego but most of the species and taxonomic diversity in the subfamily is found in Australia. It has been suggested that diversity of pollination systems may, in part, explain species diversity and permit local species packing (Goldblatt and Manning, 2006). In Australia, diversity in the Styphelioideae increases dramatically southward with southwest Western Australia and Tasmania being hotspots of both diversity and regional endemism.

Building upon my previous chapters I explore the relationships between floral traits and potential pollinator profiles. I summarise pollination syndromes for the Styphelioideae from the known plant and pollinator relationships and use them to infer pollination systems where

they are currently unknown. I test the relationships between pollination syndromes and the evolutionary history derived from the analysis in Chapter 6.

7.3 Methods

7.3.1 Potential pollinator profiles

Information on floral visitors came from the published literature and my field observations (Appendix 7-1, Tables A and B). In order to standardise the data from the different sources I used the term 'potential pollinator' (hereafter synonymous with ‘pollinator’) to describe an animal observed to interact with the flowers of an epacrid. Thus, a flower visitor was considered a potential pollinator only when it was described in the literature as *foraging at flowers, brushing anthers and stigma,* and/or *carrying epacrid pollen*.

In order to analyse and interpret plant and animal relationships, species level identification was required for plants to be included in analyses. For animals, enough information was required for them to be categorised into functional groups based on Fenster et al. (2004) – bee (Hymenoptera), fly (Diptera), beetle (Coleoptera), butterfly (Lepidoptera), moth (Lepidoptera), wasp (Hymenoptera), bird (Aves)) (Appendix 7-2).

To collate potential pollinator information from the literature, I undertook searches using Google Scholar (most recently 11 May 2011) for the key words ‘Pollinat* Epacridaceae’ or ‘Pollinat* Styphelioideae’. I also used the words ‘Pollination’, ‘Epacridaceae’ and ‘Styphelioideae’ to search within journals that routinely publish on pollination ecology including the *American Journal of Botany*, *Annals of Botany*, *Annual Review Ecology, Evolution and Systematics*, *Austral Ecology*, *Australian Journal of Botany*, *Australian Journal of Entomology*, *Australian Journal of Zoology*, *Biotropica*, *Botanical Journal of the Linnean Society*, *Cunninghamia*, *Ecology*, *International Journal of Plant Sciences*, *Journal of Experimental Botany*, *Nature*, *New Zealand Journal of Botany*, *Oecologia*, *Plant Biology*, *Plant Species Biology*, *Plant Systematics and Evolution*, *South African Journal of Botany*. It is acknowledged that each study had its own agenda, thus, the completeness of the documented pollinator profiles may be variable and may not cover all possible pollination scenarios in the epacrids. For instance, there is a notable absence of nocturnal pollination studies.

7.3.2 Floral trait profiles

Floral traits were obtained for each epacrid species where potential pollinator(s) were known. Floral trait information was also scored at the generic level, encompassing the variability within each of the 38 recognised genera and one undescribed genus, *Pseudactinia* (Appendix 7-3, Table A). All information on floral traits was obtained from the literature (Allan, 1961; Curtis, 1963; Laing and Blackwell, 1964; Van Royen, 1982; Jessop and Toelken, 1986; Harden, 1992; Corrick et al., 1996; Walsh and Entwisle, 1996; Western Australian Herbarium, 1998-; Packowska and Chapman, 2000; Wheeler et al., 2002). Information on *Cyathopsis* and the undescribed *Pseudactinia* were provided by Dr. Elizabeth Brown from the National Herbarium of New South Wales in June 2011. I scored 16 floral traits, known to be important in pollination ecology (Faegri and van de Pijl, 1979; Fenster et al., 2004), as presence/absence data and included all known variability in traits that were described for each taxa. The traits used were display colour, flower type and length, corolla throat constriction, and nectar. Traits were scored as 1 if present and 0 if not. For instance, red corolla = 1 or not red corolla = 0. Display colour was categorised as blue, purple, red, pink, orange, yellow, green, and white/cream. This usually referred to the corolla. However, the corolla-abscising *Richea* taxa present like a brush flower and previous research suggests that animal-visitation occurs after corolla abscission (Chapter 5) thus I scored them for stamen rather than corolla colour. Flower shape was scored as cup/bell, brush, long tube (10+ mm), medium tube (5 to <10 mm), short tube (< 5 mm). Corolla mouth was recorded as 'constricted' where the corolla was nearly closed at the top such as in *Astroloma* species, 'narrow' in cases where it tapered in near the top, and 'wide' where it was at least as wide as the rest of the floral tube; and nectar was recorded.

As I used the species-level floral trait data to train a classification model that was then used to identify pollination syndromes at genus level I avoided traits that were not readily available in the literature and traits that were subjective in their interpretation, for instance floral hairiness, and anther and stigma position. The traits that I have used feature widely in the pollination literature and together cover much of the obvious floral diversity observed in the epacrids. The traits, particularly display colour, have been shown to be malleable and thus have the potential to be influenced by pollinators (Whitney and Glover, 2007). However, it is probable that additional traits will prove useful for refining species-level epacrid pollination syndromes

as more data become available.

7.3.3 Data analysis

Using the subset of epacrids for which I had floral and potential pollinator information I undertook three NMDS analyses using the ecodist library in R 2.2.1 (Goslee and Urban, 2007).

Firstly, I explored the relationships between plant species based on their potential pollinator profiles. An ordination of epacrids by their biotic pollinators (categorised as the following functional groups – bee (Hymenoptera), fly (Diptera), beetle (Coleoptera), wasp (Hymenoptera), butterfly (Lepidoptera), moth (Lepidoptera), and bird (Aves)) was undertaken (Appendix 7-3, Table B). I only included native pollinators in the analyses, excluding the two introduced species, the honey bee *Apis mellifera* and bumble bee *Bombus terrestris.* I do not consider either the honeybee (introduced to Australia in the early nineteenth century) nor bumblebee (introduced in 1992 (Semmens, 1993)) as part of the historical pollinator assemblage as they are unlikely to have had time to effect evolutionary change on floral traits. *Acrotriche* species were excluded from analyses due to uncertainty regarding their potential pollinators (Johnson et al., 2011 (Chapter 3); Schneemilch et al., 2011). Lizards were excluded due to uncertainty about their role as either potential pollinators or nectar-robbers of *Richea scoparia* (Chapter 5; (Olsson et al., 2000). The two records of mosquitoes as potential pollinators were also excluded from the analyses. The Bray-Curtis dissimilarity matrix was used as input to the ordination. The potential pollinators that were significantly ($P < 0.05$) linearly related to the variation in potential pollinator profiles were fitted as vectors. Vectors indicate the direction and strength of the relationships of attributes in ordination space. *P*-values were based on 1000 permutations.

Secondly, I undertook an NMDS ordination of epacrid species based on floral traits (Appendix 7-3, Table A). The floral traits that were significantly ($P < 0.05$) linearly related to the variation in species profiles were fitted as vectors. *P*-values were based on 1000 permutations.

Thirdly, I undertook an NMDS ordination of epacrid species based on their floral traits, and the potential pollinators that were significantly ($P < 0.05$) linearly related to the variation in floral trait profile were then fitted as vectors. In ecodist vector fitting is only supported for

two dimensional ordinations. However, the 2D ordinations have a relatively high stress. Stress measures the fit of the lower dimension to the original distances. Thus, for the purpose of comparison, I have included the significant vectors obtained from the same three dimensional ordinations run in DECODA (Database for Ecological COmmunity DAta) (Minchin, 2001) using Bray-Curtis dissimilarity matrices as inputs to the ordinations. The 2D ordinations with vectors fitted are used for display purposes.

Using the Mantel test in the ecodist package in R 2.2.1 (Goslee and Urban, 2007) I tested whether the Bray-Curtis dissimilarity matrix based on potential pollinators was correlated with the Bray-Curtis matrix based on floral traits (Appendix 7-3, Tables A and B). All *P*-values are for the two-tailed test and were based on 10,000 permutations.

To test whether floral traits varied between those taxa that had bird visitors in their pollinator profiles and those taxa that did not have birds in their profiles, I used the Mantel test (in the ecodist package in R) to assess whether the Bray-Curtis matrix based on floral traits was associated with a matrix containing 0s for pairs of epacrids with birds in their pollinator profiles or pairs of epacrids without birds in their profiles, and 1s otherwise. I repeated this analysis for each of the biotic pollinator groups.

7.3.4 Identifying pollination syndromes from known relationships

Statistical classification was used to obtain a set of floral attributes that would be predictive of the pollinator groups that were found to be significantly related to floral traits by the Mantel test. Random Forests (RF) (Breiman, 2001) is a classifier capable of modeling complex interactions among predictor variables. It builds a *forest* of classification trees based on random subsets of the data, using randomly restricted and selected predictors for each of the splits in the trees (Strobl et al., 2008). Strobl et al. (2009) suggest that this enables a better examination of the contribution and behaviour of each predictor, particularly when compared with simpler models (such as simple or mixed effect regression models). In addition, results from numerous classification trees have been found to be superior to that of one classification tree (Strobl et al., 2008). RF was run using the randomForest 4.6-2 library in R2.2.1 (Breiman, 2001; Liaw and Wiener, 2002).

RF was used to build a classification model for bird pollination from the floral traits and pollinator tables of individual epacrids (Appendix 7-3A,B). The RF program built 500

classification trees, with four predictors (floral traits) tried at each split. To determine a classification for each epacrid the classifier votes over each of the 500 random trees and the majority decision is returned. About 63% of the original observations occur in each bootstrap sample. A particular strength of the RF technique is that the observations not used in a particular bootstrap sample (remaining 37%) constitute the out-of-bag (OOB) observations and can be used to estimate both classification error and the importance of each variable (Cutler et al., 2007). Variable importance is measured by randomly permuting the OOB observations and passing them down the trees to get new predictions. It is the difference between the misclassification rate for the randomly permuted OOB and the original OOB data, divided by the standard error (Cutler et al., 2007). This process returned a relative ranking of the variable importance of the floral traits in predicting bird pollination. Classification models were also built for fly and bee pollination. Thus, the pollination syndromes obtained here, related very specifically to the subset of data from the Styphelioideae. Using two-way Chi-square analyses in MINITAB 15, I tested the individual significance of each of the four most important floral trait variables given by the RF analyses for bird, fly and bee.

To test that the bird, fly and bee RF classification models were functioning accurately I used each of them to predict pollination systems from the floral traits data set that was used to build them. The aim was to get as close to 100% accuracy rate as possible. The bird classification model returned 80% of the original species known to be bird-visited, the fly model 83% and the bee model 74%. In addition, the fly model returned 11 species not known to have flies as pollinators and the bee model four. However, I found that by creating a model for fly and bee together the accuracy improved to 91%, with seven additional species predicted. Although the bird model was unable to predict five of the known species, it did not return any additional species.

7.3.5 Inferring pollination syndromes where they are unknown

I used the classification models for bird and combined fly and bee developed above to identify pollination syndromes from a data set consisting of the floral traits for each genus (Appendix 7-3, Table C). Genus-level was used to minimise the size of the floral traits data set. So that the genus-level data set bore a greater resemblance to the species-level data set the classification models were trained on, I examined the genus data for genera that contained

multiple flower colours, flower shapes and corolla lengths, and/or corolla mouth constriction. I then used the literature to determine how these genera could be split into more than one unit that recognised the main colours and morphological differences between the species. For instance, *Trochocarpa* contained species with red, pink and white flowers, small tubed or cup-shaped flowers. *Trochocarpa* is now entered in the data set as *Trochocarpa*A: red or pink flowers with short floral tubes (e.g. *T. cunninghamii* and *T. thymifolia*); and *Trochocarpa*B: white cup-shaped flowers (e.g. *T. gunnii*, *T. bellendenkerensis*, *T. laurina*). Appendix 7-4 explains the splits that have been made.

7.3.6 Evolutionary context

I mapped potential pollinators onto the genus-level Bayesian phylogenetic tree presented in Chapter 6 (Fig. 6-2). For clarity I have reduced the full plant species labels found on the original figure in Chapter 6 to genus only. I mapped the floral traits (Allan, 1961; Curtis, 1963; Laing and Blackwell, 1964; Van Royen, 1982; Jessop and Toelken, 1986; Harden, 1992; Corrick et al., 1996; Walsh and Entwisle, 1996; Packowska and Chapman, 2000; Western Australian Herbarium, 1998-; Wheeler et al., 2002) and the known occurrences of bird, insect, and wind pollination according to Ladd (2006), and the subset of bee pollination referred to as buzz pollination (Houston and Ladd, 2002; Johnson and McQuillan, 2011 (Chapter 4)) onto the phylogeny. Inferred pollinators identified through the RF prediction process outlined above, were also mapped onto the tree.

A number of genera are paraphyletic: *Astroloma*, *Conostephium*, *Brachyloma*, *Leucopogon*, *Styphelia*, *Epacris*, *Dracophyllum*, and *Richea*. To determine the floral traits at each separate location of these genera, I referred to previous phylogenetic studies that targeted specific groups of epacrids (Powell et al., 1997; Crayn and Quinn, 2000; Quinn et al., 2003; Wagstaff et al., 2010). Although I have not included the floral traits of species whose phylogenetic position is unknown with respect to the paraphyly, the species included cover the major variation known for each of these genera.

As red flowers were important predictors of bird, fly and bee visitation, I tested the hypothesis that their distribution was random with respect to the tree. The test statistic was the parsimony score of the character ‘red flowers’ (that is, the number of times that the change is made from red to not red or vice versa) on the 3 gene majority rule tree (Chapter 6 Fig. 6-2). The

character ‘red flowers’ was then randomised with respect to species labels and shuffled 1000 times and the parsimony scores recorded. Using the same hypothesis, I also tested nectarless flowers and white flowers.

7.4 Results

7.4.1 Potential pollinator profiles

Information on potential biotic pollinators was available for 87 epacrid species representing 22 genera and six tribes (Appendix 7-2). This represented about 15% of all species in the Styphelioideae. Six of the seven animal groups showed a significant influence on the variation among potential pollinator profiles of the epacrid species in the ordinations: fly, bee, beetle, butterfly, bird, and moth (Figs. 7-1 & 7-2, Table 7-1). Most of the epacrids with birds in their pollinator profiles were clustered in ordination space. In contrast, the epacrids associated with the various insect groups tended to be scattered across the plot with smaller hubs of taxa occupying the same spaces, thus reflecting a general dissimilarity in pollinator profiles. The vectors for bird and beetle pollination were opposed to each other, as were those for bee and moth pollination. About 41% of epacrids had bees in their pollinator profiles, 35% flies, 29% birds, 26% butterflies, 22% beetles, 15% moths and 6% wasps. Only, 3% of taxa had both birds and insects in their profiles. One-third of epacrids (35%) used more than one pollen-vector and 26% had both bees and flies in their profile (Fig. 7-2, Appendix 7-2). Flies and bees accounted for the greatest number of potential pollinating taxa and visited the most plant species (Appendix 7-2).

Figure 7-1 Epacrid diversity: A. Bird-pollinated flowers of *Prionotes*; B. Hoverfly (Syrphidae) on *Leucopogon parviflorus*, C. Beetles (*Chauliognathus tricolor*) on *Richea scoparia*; D. Macleay's swallowtail butterfly (*Graphium macleayanum*) visiting *Dracophyllum minimum*; E. Bee (*Exoneura* sp.) collecting pollen by sonication from *Sprengelia incarnata*; F. *Richea scoparia* – orange flowers are rare in epacrids; G. Native bee collecting pollen from *Epacris marginata*; H. *Acrotriche serrulata* flowers with secondary pollen presentation

Table 7-1 Significance of vectors for 2 and 3 dimensional ordinations (* = significant at P = 0.001 level, ** P = 0.01, * P = 0.05; bold type indicates there was a difference between 2D and 3D vectors at 0.05 level; ordination stress for epacrids by pollinators 2D = 0.216, 3D = 0.034; and for epacrids by floral traits 2D = 0.219, 3D = 0.141)**

	2D ordination		3D ordination	
	r	*P*	r	*P*
Ordination of epacrids by pollinators – significance of pollinators fitted as vectors				
Fly	0.660	0.001***	0.784	<0.001***
Bee	0.719	0.001***	0.749	<0.001***
Beetle	0.535	0.001***	0.423	0.001***
Butterfly	0.481	0.001***	0.739	<0.001***
Bird	0.815	0.001***	0.869	<0.001***
Moth	0.334	0.001***	0.541	<0.001***
Wasp	0.148	0.289	0.259	0.117
Ordination of epacrids by floral traits - significance of pollinators fitted as vectors				
Fly	0.431	0.002**	0.481	<0.001***
Bee	0.449	0.001***	0.485	<0.001***
Beetle	0.166	0.303	0.269	0.093
Butterfly	0.287	0.024*	0.352	0.011*
Bird	0.568	0.001***	0.612	<0.001***
Moth	0.125	0.535	0.185	0.396
Wasp	0.279	0.057*	0.296	0.052
Ordination of epacrids by floral traits - significance of floral traits fitted as vectors				
Blue corolla	0.233	0.053	0.245	0.104
Purple corolla	**0.225**	**0.110**	**0.321**	**0.032***
Red corolla	0.225	0.001***	0.792	<0.001***
Pink corolla	**0.154**	**0.345**	**0.406**	**0.001*****
White corolla	0.654	0.001***	0.737	<0.001***
Green corolla	**0.171**	**0.288**	**0.364**	**0.013***
Yellow corolla	0.535	0.001***	0.489	<0.001***
Brush flower	0.628	0.001***	0.718	<0.001***
Long tube	0.731	0.001***	0.749	<0.001***
Medium tube	0.457	0.001***	0.592	<0.001***
Short tube	0.462	0.001***	0.675	<0.001***
Bell/cup flower	0.610	0.001***	0.690	<0.001***
Constricted corolla	0.645	0.001***	0.643	<0.001***
Narrow corolla	0.630	0.001***	0.651	<0.001***
Wide corolla	0.822	0.001***	0.825	<0.001***
Nectar	0.509	0.001***	0.522	<0.001***

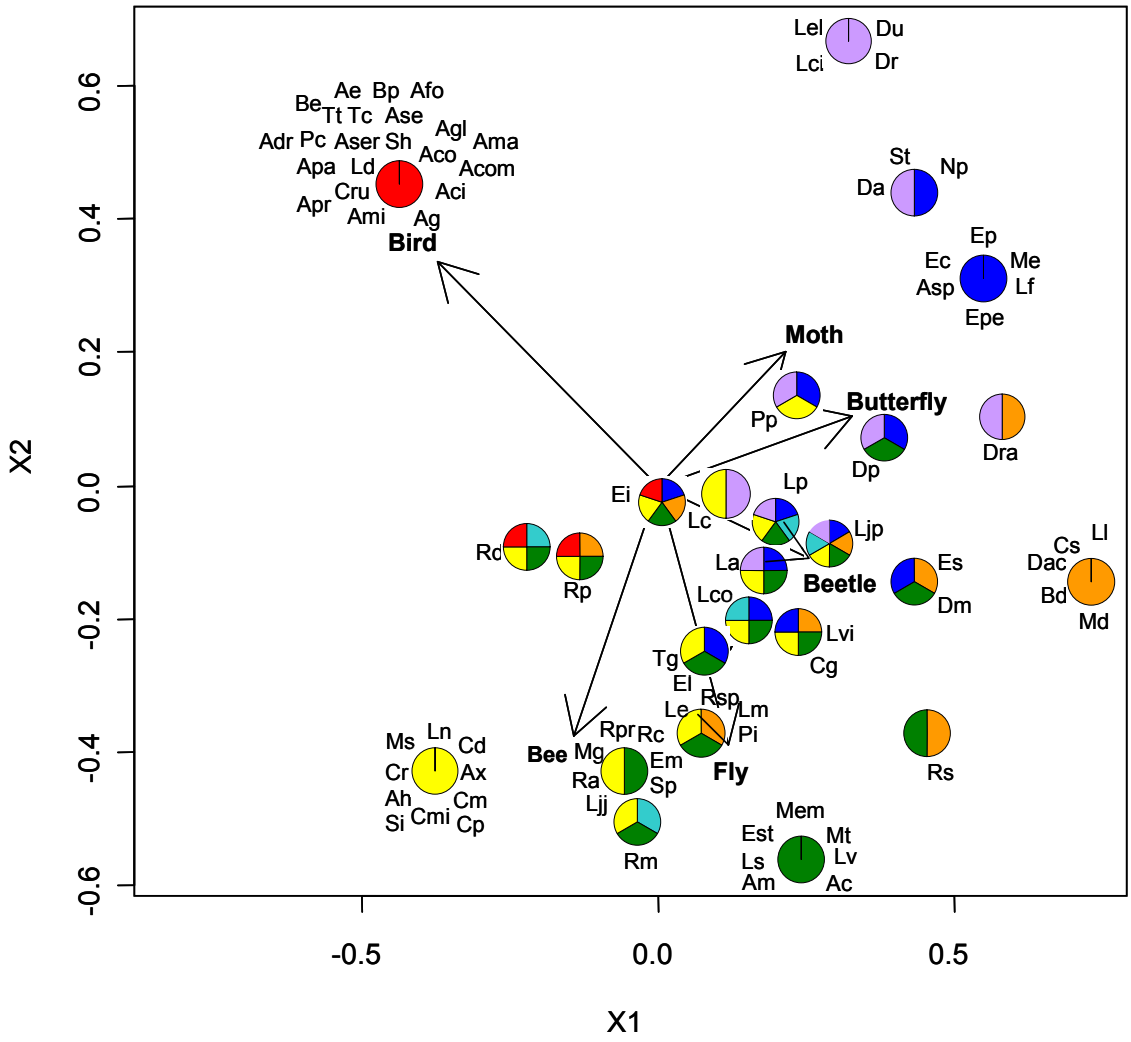


Figure 7-2 Ordination of epacrids according to potential pollinator profiles (Bird, Fly, Bee, Beetle, Butterfly, Moth, Wasp). Attributes that were significant (P < 0.05) predictors of the variation between species are fitted as vectors. Stress in 2D = 0.216. Plant species codes and full names given over page.

Figure 7-2 continued: Species codes and names

Code	Species name	Code	Species name	Code	Species name
Ac	*Acrotriche cordata*	Cs	*Cyathodes straminea*	Lp	*Leucopogon parviflorus*
Aci	*Astroloma ciliatum*	Da	*Dracophyllum acerosum*	Ls	*Lissanthe strigosa*
Aco	*Astroloma conostephioides*	Dac	*Dracophyllum acicularifolium*	Lv	*Leucopogon verticillatus*
Acom	*Astroloma compactum*	Dm	*Dracophyllum minimum*	Lvi	*Leucopogon virgatus*
Adr	*Astroloma drummondii*	Dp	*Dracophyllum pronum*	Md	*Montitega dealbata*
Ae	*Astroloma epacridis*	Dr	*Dracophyllum rosmarinifolium*	Me	*Monotoca elliptica*
Afo	*Astroloma foliosum*	Dra	*Dracophyllum ramosum*	Mem	*Monotoca empetrifolia*
Ag	*Andersonia grandiflora*	Du	*Dracophyllum uniflorum*	Mg	*Monotoca glauca*
Agl	*Astroloma glaucescens*	Ec	*Epacris corymbifolia*	Ms	*Monotoca submutica*
Ah	*Andersonia heterophylla*	Ei	*Epacris impressa*	Mt	*Dielsiodoxa tamariscina*
Am	*Andersonia micrantha*	El	*Epacris lanuginosa*	Np	*Needhamiella pumilio*
Ama	*Astroloma macrocalyx*	Em	*Epacris marginata*	Pc	*Prionotes cerinthoides*
Ami	*Astroloma microcalyx*	Ep	*Epacris paludosa*	Pi	*Pentachondra involucrata*
Apa	*Astroloma pallidum*	Epe	*Epacris petrophila*	Pp	*Pentachondra pumila*
Apr	*Astroloma prostratum*	Es	*Epacris serpyllifolia*	Ra	*Richea acerosa*
Ase	*Andersonia setifolia*	Est	*Epacris stuartii*	Rc	*Richea contentinalis*
Aser	*Astroloma serratifolium*	La	*Leucopogon australis*	Rd	*Richea dracophylla*
Asp	*Andersonia sprengeloides*	Lc	*Leucopogon capitellatus*	Rm	*Richea milliganii*
Ax	*Astroloma xerophyllum*	Lci	*Lysinema ciliatum*	Rp	*Richea pandanifolia*
Bd	*Brachyloma daphnoides*	Lco	*Leucopogon collinus*	Rpr	*Richea procera*
Be	*Brachyloma ericoides*	Ld	*Leptecophylla divaricata*	Rs	*Richea scoparia*
Bp	*Brachyloma preissii*	Le	*Leucopogon ericoides*	Rsp	*Richea sprengelioides*
Cd	*Conostephium drummondii*	Lel	*Lysinema elegans*	Sh	*Styphelia hainesii*
Cg	*Cyathodes glauca*	Lf	*Lysinema fimbriatum*	Si	*Spengelia incarnata*
Cm	*Coleanthera myrtoides*	Ljj	*Leptecophylla juniperina* var. *juniperina*	Sp	*Spengelia propinqua*
Cmi	*Conostephium minus*	Ljp	*Leptecophylla juniperina subsp. parvifolia*	St	*Styphelia tenuifolia*
Cp	*Conostephium pendulum*	Ll	*Leucopogon lanceolatus*	Tc	*Trochocarpa cunninghamii*
Cr	*Conostephium roei*	Lm	*Leucopogon montanus*	Tg	*Trochocarpa gunnii*
Cru	*Cosmelia rubra*	Ln	*Leucopogon nutans*	Tt	*Trochocarpa thymifolia*

7.4.2 Relationships between floral traits and pollinator profiles

Four of the pollinators showed a significant influence on the variation among floral trait profiles of epacrid species. Twelve and 15 floral traits showed a significant influence on the variation among floral trait profiles of epacrid species in the 2D and 3D ordinations respectively. Differences between the ordinations were the significance of purple, pink and green corollas (Table 7-1, Fig. 7-3). With the exception of some of the smaller white flowered species that shared the same ordination space, the epacrids were scattered across the plot reflecting a general dissimilarity in floral profiles. Red flowers were closely associated with long floral tube lengths, and were opposed to white flowers which were associated with wide corolla mouths. The vector for bird visitation shared the same direction as red flowers and long floral tubes, and was opposed to the vectors for fly and bee visitation that shared a similar location. The vectors for the insect visitors were loosely clustered.

The Mantel test showed that there was a significant relationship between floral trait profiles and pollinator profiles ($r = 0.175$, $P = 0.0001$). Floral trait profiles were significantly related to bird visitors ($r = 0.278$, $P = 0.001$), fly visitors ($r = 0.123$, $P = 0.001$), and bee visitors ($r = 0.072$, $P = 0.005$). There were no significant relationships between floral trait profiles and beetle, butterfly, moth and wasp visitors.

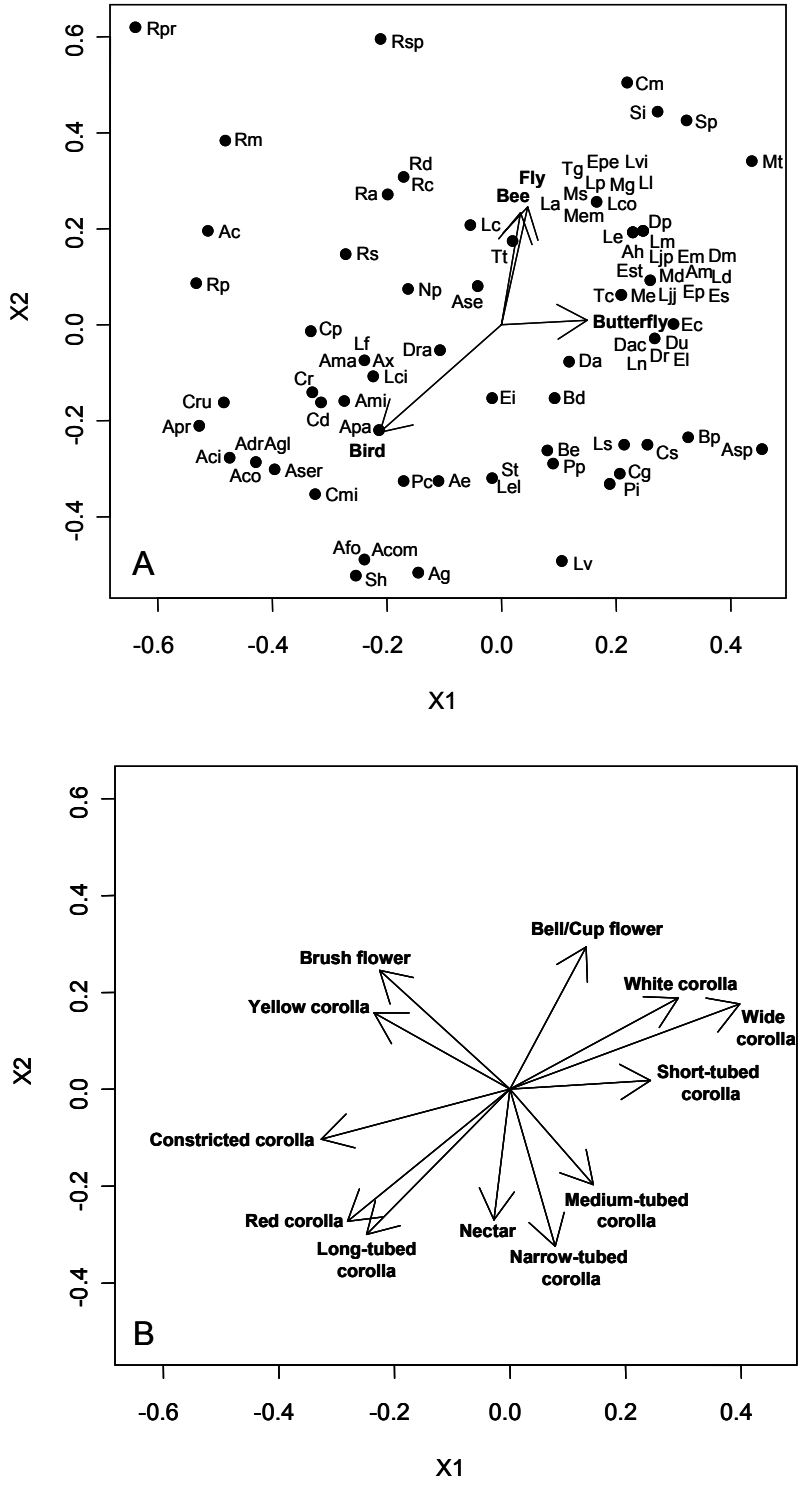


Figure 7-3 Ordination of epacrids according to floral trait profiles. Stress in 2D = 0.219. Potential pollinator groups that were significant ($P < 0.05$) predictors of the variation between species are fitted as vectors. B. The floral attributes that were significant ($P < 0.05$) predictors of the variation between species are fitted as vectors and shown in a separate plot for clarity. For species names refer to Fig. 7-2.

7.4.3 Identifying pollination syndromes from known relationships

Red flowers were the most important predictor variable for a bird-visited epacrid and the most important predictor variable of pollinator-type overall. For a bird-visited plant the most important predictor variables were the presence of red flowers, long floral tubes, and the absence of white flowers. Although less important, an absence of pink flowers was the fourth predictor variable. Conversely, the absence of red flowers and long floral tubes were in the top five predictors of both fly- and bee-visited plants. Brush flowers were important predictors for both, while bell/cup-shaped flowers were important for bees. The absence of constricted corollas and medium floral tubes were important for flies. Thus, the bird pollination syndrome was readily distinguished from bee and fly syndromes. However, bee and fly pollination syndromes were less readily distinguished from each other. The RF classification predicted bird pollination with accuracy of about 86%, while fly and bee pollination were 78% and 72% respectively (Figs. 7-4 to 7-6).

When traits were assessed individually I found that for bird visitation, the presence of red flowers and long floral tubes were significant ($\chi^2 = 39.298$, DF = 1, $P<0.001$; $\chi^2 = 23.286$, DF = 1, $P<0.001$ respectively), as was the absence of white flowers ($\chi^2 = 24.638$, DF = 1, $P<0.001$). Pink flowers were not significantly related to bird visitation ($\chi^2 = 1.650$, DF = 1, $P = 0.199$).

For fly visitation, the absence of red flowers, long floral tubes and constricted corollas were significant ($\chi^2 = 13.954$, DF = 1, $P<0.001$, $\chi^2 = 15.843$, DF = 1, $P<0.001$, $\chi^2 = 15.843$, DF = 1, $P<0.001$ respectively), as was the presence of brush flowers ($\chi^2 = 13.733$, DF = 1, $P<0.001$). Medium floral tubes were not significantly related to fly visitation ($\chi^2 = 1.664$, DF = 1, $P = 0.197$).

For bee visitation, the presence of bell/cup shaped flowers or brush flowers were significant ($\chi^2 = 6.627$, DF = 1, $P = 0.010$; $\chi^2 = 8.187$, DF = 1, $P = 0.004$ respectively). The absence of red flowers and long floral tubes were also significantly associated with bee visitation ($\chi^2 = 7.425$, DF = 1, $P = 0.006$; $\chi^2 = 11.655$, DF = 1, $P<0.001$).

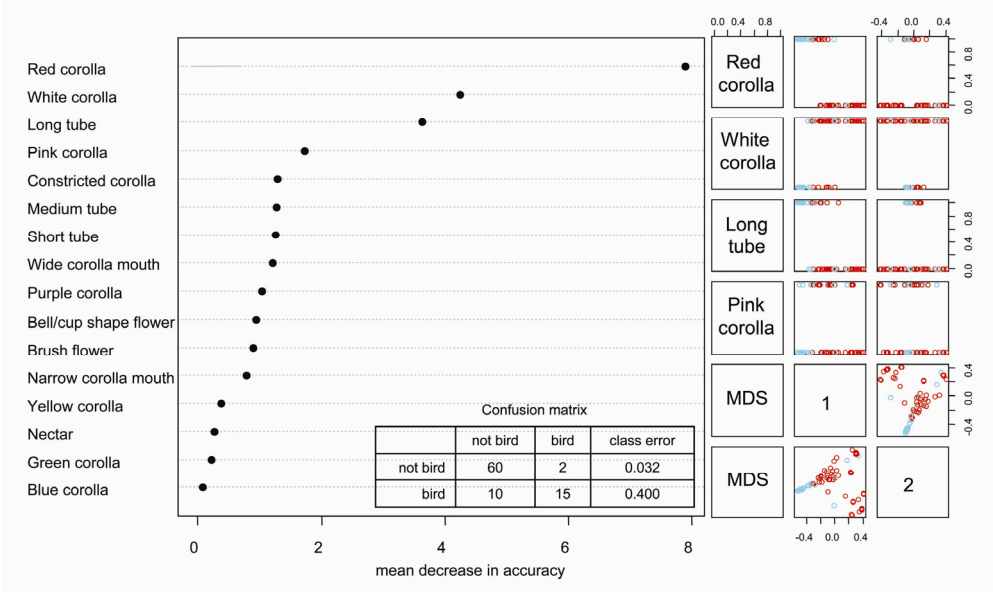


Figure 7-4 Variable importance for predictor variables from RF classifications used for predicting bird-visited epacrid taxa (out-of-bag estimate of error rate: 13.79%). Top four rows of matrix shows detail for four most important variables where blue denotes bird and red denotes not bird, and the y-axis of the matrix is 0 = no and 1 = yes; and the bottom two rows show the RF 2D multi-dimensional scaling. In confusion matrix: columns = prediction outcomes; rows = known values.

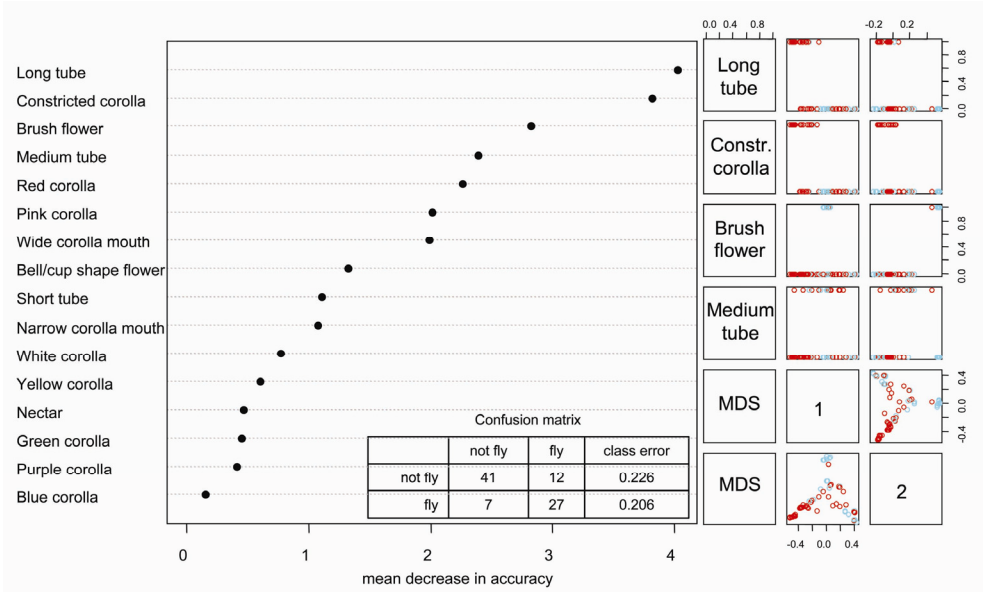


Figure 7-5 Variable importance for predictor variables from RF classifications used for predicting fly-visited epacrid taxa (out-of-bag estimate of error rate: 21.84%). Top four rows of matrix shows detail for four most important variables where blue denotes fly and red denotes not fly, and the y-axis of the matrix is 0 = no and 1 = yes; and the bottom two rows show the RF 2D multi-dimensional scaling. In confusion matrix: columns = prediction outcomes; rows = known values.

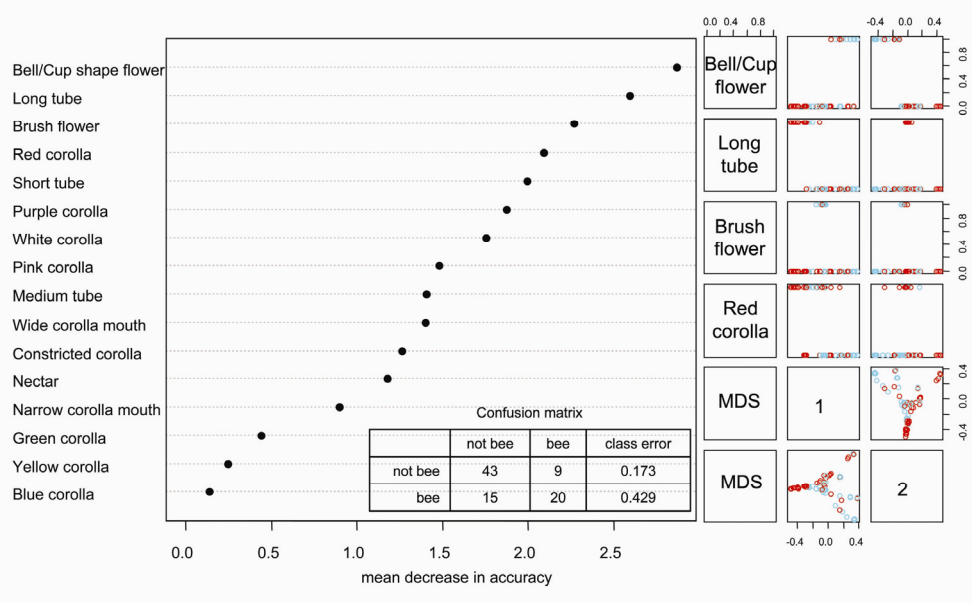


Figure 7-6 Variable importance for predictor variables from RF classifications used for predicting bee-visited epacrid taxa (out-of-bag estimate of error rate: 27.59%). Top four rows of matrix shows detail for four most important variables where blue denotes bee and red denotes not bee, and the y-axis of the matrix is 0 = no and 1 = yes; and the bottom two rows show the RF 2D multi-dimensional scaling. In confusion matrix: columns = prediction outcomes; rows = known values.

7.4.4 Inferring pollination syndromes where they are unknown

Using the classification models, bird and insect (fly and bee) pollination systems were inferred for most genera (Table 7-2; Appendix 7-5). The classification model for bird pollination predicted 10 genera, including 80% of the currently known bird-pollinated genera. The only known bird-pollinated genera that were not predicted were *Leptecophylla* and *Brachyloma.* Five additional genera were found to have a bird pollination syndrome. Four of these are included in Table 7-2. *Conostephium* has not been included as it has been convincingly shown to be buzz-pollinated (Houston and Ladd, 2002). The fly and bee classification model predicted 88% of known fly- and bee-pollinated genera. The only two known genera not predicted were *Cyathodes* and *Pentachondra.* Fifteen additional genera were found to have a fly and/or bee pollination syndrome.

Table 7-2 Predicted epacrid pollination systems

Pollinator type	Genera known to use pollinator type in at least one species	Additional genera predicted to be visited by pollinator-type
Bird	*Andersonia* *Astroloma* *Brachyloma* *Cosmelia* *Epacris* *Leptecophylla* *Prionotes* *Richea* *Styphelia* *Trochocarpa*	*Archeria* *Conostephium* *Cyathopsis* *Leucopogon* *Melichrus*
Fly and bee	*Acrotriche* *Andersonia* *Astroloma* *Coleanthera* *Conostephium* *Cyathodes* *Dielsiodoxa* *Dracophyllum* *Epacris* *Leptecophylla* *Leucopogon* *Lissanthe* *Monotoca* *Pentachondra* *Richea* *Sprengelia* *Trochocarpa*	*Acrothamnus* *Agiortia* *Androstoma* *Archeria* *Brachyloma* *Budawangia* *Decatoca* *Lebetanthus* *Melichrus* *Montitega* *Needhamiella* *Oligarrhena* *Planocarpa* *Pseudactinia* *Rupicola*

7.4.5 Evolutionary context

While insect pollination appears widespread across the tree occurring in 50 out of a possible 58 locations, bird pollination is known or predicted from just 16 locations (Fig. 7-7). Bird pollination is known from the basal tribe Prionoteae, and insect pollination is also predicted for this tribe. Insect pollination is associated with all seven tribes and bird pollination is associated with all but Oligarrheneae. No known or predicted pollination system information is available for *Croninia* and *Woollsia.*

Nectarless flowers are known from seven locations on the tree, across four tribes. With the exception of *Budawangia*, nectarless flowers coincide, or are predicted to coincide with either wind or buzz pollination systems. Wind pollination appears twice in the paraphyletic *Richea*.

White flowers occur at 52 locations in the tree, pink flowers at 25, red flowers at 19, yellow flowers at nine, green flowers at seven, purple flowers at five, and orange and blue flowers at one. White flowers are widespread across the tree, while the other colours are more sporadic. Multiple flower colours occur at 32 locations, with three or more colours present at 18 locations. Short floral tubes occur at 29 locations, medium tubes at 18 locations, long tubes occur at 17, bell/cup shaped flowers at 11, and brush flowers at three. Wide corolla mouths occur at 33 locations, and constricted corolla mouths at 16, and narrowed corolla mouths at 12 locations.

The hypotheses that the distributions of red flowers, white flowers, and nectarless flowers are random with respect to the tree were not rejected (Table 7-3).

Table 7-3 Distribution of parsimony scores from 1000 shuffles (* = actual number of observed changes)

Red flowers		White flowers		Nectarless flowers	
No. of changes	Frequency	No. of changes	Frequency	No. of changes	Frequency
19	81	8	781	7	815
18	220	*7	193	*6	172
17	280	6	26	5	13
*16	251	5	0	4	0
15	103	4	0	3	0
14	44	3	0	2	0
13	17	2	0	1	0
12	3				
11	1				

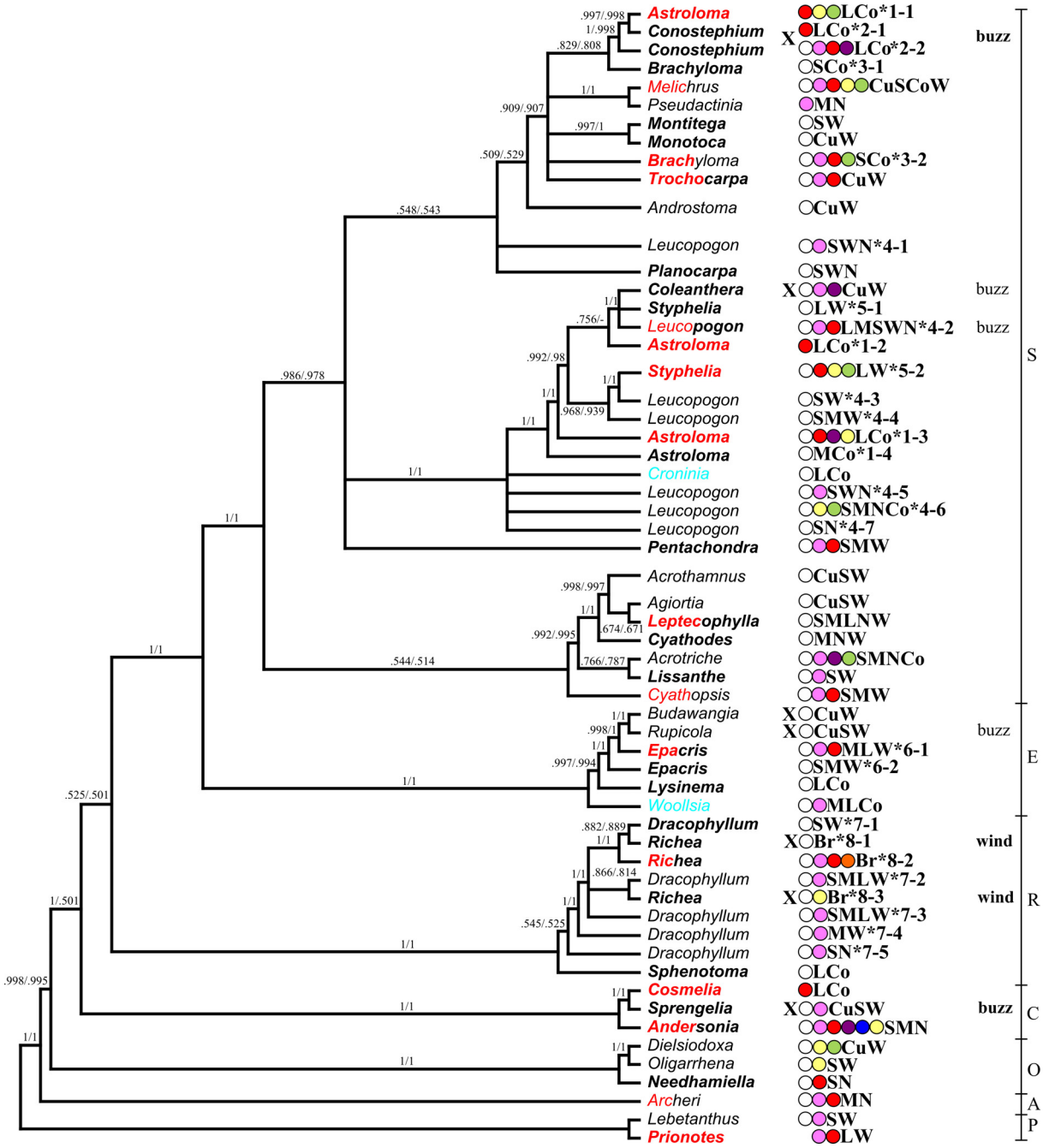


Figure 7-7 Evolution of pollination systems of epacrids (bold type = known pollination system, normal type = inferred pollination system, red type = bird pollination, black type = insect pollination (e.g. *Andersonia* = bird and insect pollination present in genus), light blue = no prediction; circles contain flower colour, S = short tube, M = medium tube, L = long tube, Cu = bell/cup flower, W = wide corolla mouth, N = narrow corolla mouth, Co = constricted corolla mouth; wind = at least one species with wind pollination present, buzz = at least one species with buzz pollination;*numbers = paraphyletic genus. For *Astroloma* all groups are based on the relationships given in Quinn et al. (2003): 1-1 = *A. conostephioides*, *A. pinifolium*, *A. baxteri*; 1-2 = *A. recurvum*, *A. epacridis*, *A. humifusum*; 1-3 = *A. ciliatum*, *A. macrocalyx*, *A. pallidum*; *A. xerophyllum*. For *Conostephium*: 2-1 = *C. pungens* and 2-2 = *C. pendulum* (Chapter 6, Fig.

6-2). For *Brachyloma*: 3-1 = *Brachyloma*B (Powell et al., 1997); 3-2 = group B1 (Quinn et al., 2003) and *Brachyloma*A plus *B. scortechinii* (Powell et al., 1997). For *Leucopogon*: 4-1 = species included in group L1 (Quinn et al., 2003) plus *L. gibbosus* (Chapter 6); 4-2 = *L. oxycedrus*, *L. propinquus*, *L. allittii*, *L. nutans*, *L. pendulus*, *L. cuneifolius* (part of As group in Quinn et al., 2003); 4-3 = traits from *Leucopogon esquamatus* (part of As group in Quinn et al., 2003); 4-4 = traits from *L. fraseri*NSW, *L. neoanglicus*, *L. setiger*, *L. fraseri*NZ (part of As group in Quinn et al., 2003); 4-5 = *L. muticus*, *L. ericoides*, *L. leptospermoides* (part of L2 group in Quinn et al., 2003); 4-6 = *L. crassifolius*, *L. pogonocalyx*, *L. cymbiformis*, *L. appressus* (part of L2 group in Quinn et al., 2003); 4-7 = *L. blepharolepis*. For *Styphelia* (Quinn et al. 2003): 5-1 = *S. exarrhena*, *S. tubifolia*, *S. viridis*; 5-2 = *S. tenuifolia*, *S. exserta.* For *Epacris*: 6-1 = *E. impressa*, 6-2 = *E. lanuginosa* (Crayn and Quinn, 2000). For *Dracophyllum* (7-1 to 7-5) and *Richea* (8-1 to 8-3) groups refer to the parpahyly in Wagstaff et al. (2010). Phylogenetic relationships are from the Bayesian analysis in Chapter 6 (Fig. 6-2). P = Prionoteae, A = Archerieae, O = Oligarrheneae, C = Cosmelieae, R = Richeeae, E = Epacrideae, S = Styphelieae.

7.5 Discussion

Floral trait profiles in the epacrids were related to bird, fly and bee visitors. For bird-visited epacrids, similar phenotypic traits were observed in separate parts of the evolutionary tree. Thus, a pollination syndrome for ornithophily in epacrids can be recognised. Floral trait profiles related to fly and bee visitors overlapped in their important predictor variables. Thus, an insect pollination syndrome, based partly on the absence of floral features related to bird visitation, was identified. Red flowers and long floral tubes appeared to play a major role in either insect deterrence and/or bird attraction (Cronk and Ojeda, 2008). Given that birds visit the white flowers of *Leptecophylla* and *Brachloma* it seems that red is not necessary to attract birds. In general, red flowers are difficult for bees to detect (Cronk and Ojeda, 2008). The widespread distribution of the insect pollination syndrome across the evolutionary tree had a pattern suggestive of inheritance. Floral trait profiles were not related to beetle, butterfly, moth, and wasp visitors. This indicates either a lack of floral adaptation to these pollen vectors or a need for more information on the range of epacrids visited by these animal groups. The bird and insect classification models did not allocate the generally white, long tubed and constricted corollas of *Woollsia* and *Croninia* to either pollination syndrome.

A number of patterns were observed in the evolution of floral traits and pollination systems. The most compelling was for the convergent evolution of flower colours, except white.

According to the classification model, red colouration is the most important predictor of flowers that are likely to be visited by birds. Red flowers are spread across the evolutionary tree, appearing in 16 locations, and they have no single common ancestor - their random distribution with respect to the tree is suggestive of either random genetic mutation or selection. However, their strong relationship with birds supports evolution as a result of similar selection pressures exerted at the tips of the tree. In addition to red flowers, the presence of long floral tubes was also an important predictor of bird pollination. Thus, *Prionotes*, *Cosmelia*, and many *Astroloma* flowers conform to the bird-pollinated epacrid template. Certainly, these are genera well-known for their association with birds (Paton and Ford, 1977; Ford et al., 1979; Keighery, 1996; Wheal and 1996; Johnson et al., 2010 (Chapter 2)). On Chiloé Island, hummingbirds have long been recognised as critical for the reproductive success of plants with large, red and tubular flowers (Armesto et al., 1996). Furthermore, the association of red flowers and long floral tubes with bird pollination lends support to the traditional bird pollination syndrome (Faegri and van de Pijl, 1979). Other variations on this theme, however, are the red but short or medium tubed flowers of *Trochocarpa* and *Andersonia* species (Keighery, 1996). Faegri and van der Pijl (1979) suggest that while there may be an identifiable syndrome associated with pollination-type, in any given case, any feature belonging to the syndrome may be missing.

In the epacrids, red and long-tubed flowers are most consistently associated with bird pollen vectors, however, honeyeaters also act as pollen vectors for a wider array of flower types, including the brush-like flowers of *Richea* (Chapter 5) and the smaller and lighter-coloured flowers of some *Brachyloma* and *Leptecophylla* (Higham and McQuillan, 2000). Similarly, hummingbirds are mostly associated with red flowers, but they also pollinate the small, white, urceolate ericad, *Disterigma stereophyllum* (Navarro et al., 2008). In New Zealand, birds may play a wider role in the pollination of the flora than suggested by the traditional pollination syndromes. Honeyeaters feed at the small apparently entomophilous flowers of *Pittosporum crassifolium*, *P. eugenioides*, *Pseudpanax arboreus*, *Dysoxylum spectabile*, and *Geniostemon rupestre*, however, the nectar produced by these flowers has been found to be sufficient to sustain birds (Castro and Robertson, 1997). In contrast, the smallest white and cup-shaped flowers of the epacrids, such as those of *Monotoca*, have no record of bird visitation. Non-pollinator mediated selection pressures on a flower may include regional variation in pollinator communities, and an influence of historical contingency (Fenster et al., 2004;

Strauss and Whittall, 2006). It is probable that epacrid flowers reflect all of these influences. However, while the bird pollination syndrome identified for the epacrids may not be universally applicable, within the available Styphelioideae data it has an 86% success rate.

Given that bees and flies were found to forage at many of the same epacrids, it was unsurprising to find that they shared three of their top five most important predictor variables: an absence of long floral tubes and red flowers, and the presence of brush flowers. Although these traits were high on the list of predictors for bees, in general there was a lack of any particularly strong predictors with the importance of individual floral attributes gradually tailing off. For flies, the absence of long floral tubes and constricted corollas were notably stronger predictors than the rest. The classification model placed the absence of medium floral tubes slightly higher in importance as a predictor of fly visitation than the absence of red flowers. However, when statistically tested, the absence of red flowers was significant while medium floral tubes were not. Unlike most traditional statistical methods, when using the Random Forests classifier the importance of a variable is due to its complex interactions with other variables (Liaw and Wiener, 2002), making it a particularly useful tool for generating hypotheses and for use in conjunction with methods that produce a *P*-value. In a global test of the utility of the traditional pollination syndromes Ollerton et al. (2009) found that bee and fly pollinated plants were accurately predicted more frequently than other syndromes. Unlike the bird-visited flowers, colour was not a positive predictor variable for either of the insect groups, with the absence of red flowers being the first reference to floral colour in either predictor profile. In particular, flies and bees appear to be important pollinators of the smaller epacrid flowers that tend not to be red. Flies may be especially important pollinators under certain environmental conditions because, unlike the more periodic occurrence of bees, they are present all year and cover a greater altitudinal range (Hingston and McQuillan, 2000).

Bees are perhaps the best-adapted insect for the process of pollination (Faegri and van de Pijl, 1979), filling not only a more generalist role but also the niche-role of buzz pollinating the nectarless flowers of *Sprengelia incarnata* and *Conostephium pendulum* (Houston and Ladd, 2002; Johnson and McQuillan, 2011(Chapter 4)) and potentially *Coleanthera* and *Rupicola* (Houston and Ladd, 2002). Although *Leucopogon* flowers contain nectar, a number of species have been identified as candidates for buzz pollination due to their particularly dry pollen (Houston and Ladd, 2002). The potentially buzz-pollinated *Leucopogon nutans* and

L. pendulus occur as sister group to *L. oxyedrus* (Houston and Ladd, 2002; Quinn et al., 2003) which is red-flowered and predicted to be bird-pollinated. This group of Western Australian *Leucopogon* species has unusual diversity in pollination syndromes for the genus. Generally, nectarless flowers coincide with buzz-pollinated plants but also with the use of wind as a pollen vector in *Richea procera*, *R. sprengelioides*, and *R. victoriana* (Houston and Ladd, 2002; Ladd, 2006). Furthermore, both wind and buzz-pollinated taxa are associated with easily mobilised dry pollen (Houston and Ladd, 2002; Ladd, 2006; Johnson and McQuillan, 2011 (Chapter 4); Chapter 5). Wind pollination has a number of other lineages in the Ericaceae, including the Empetreae, and a number of *Erica* species (Stephens, 2004). There is currently no empirical evidence to suggest that birds visit any of the nectarless epacrids.

Similar to my findings, Ollerton et al. (2009) found that beetle and moth pollination were the least predicted syndromes globally. The traditional beetle pollination syndrome consists of dull flowers that are frequently green or white, open and easy to access, and that have their sexual organs exposed (Faegri and van de Pijl, 1979). Beetles visited at least eight genera of epacrid including those with white, pink and red flowers, small to medium tubular flowers, hairy or glabrous flowers, and brush flowers. Beetles such as *Chauliognathus tricolor* were found to enter the small to medium tubular flowers of plants such as *Dracophyllum minimum* and *Epacris serpyllifolia*, but also to clamber over the brush-like flowers of *Richea* species (Chapter 5). Thus, beetles that foraged on epacrids tended to visit a broader range of floral morphologies than the traditional syndrome suggested.

Although moths have been found to visit epacrids including *Dracophyllum* (Primack, 1983; Newstrom and Robertson, 2005) *Styphelia*, *Leucopogon* and *Lysinema* (Keighery, 1996) there has generally been a dearth of nocturnal pollination studies. This is likely to have a particular impact on our knowledge of moth behaviour in relation to epacrids. For example, in New Zealand some flowers have been found to be visited at night by moths, and by birds during the day (Newstrom and Robertson, 2005). Thomson (1926) observed moths and occasionally birds to visit *Dracophyllum longifolium.* Based on current information, epacrids that include moths in their visitor profiles are similar to those with butterflies. This is inconsistent with the traditional pollination syndromes that describe the flowers associated with each as quite different (Faegri and van de Pijl, 1979). Furthermore, while Faegri and van der Pijl (1979) suggest that butterflies tend to forage at vividly-coloured flowers, I have found

that in the epacrids they are also forage on white flowers. Although there was no relationship found between floral traits and butterfly visitation, it is possible that specific butterfly taxa may tend towards particular floral types. For instance, I sampled Macleay's swallowtail butterfly foraging at small to medium tubed white epacrids including *Dracophyllum minimum* and *Epacris serpyllifolia* in alpine environments and *E. corymbifolia* in buttongrass moorlands. Even though other sympatric epacrids were in flower in both these environments, including *Richea scoparia* in the alpine, and *Sprengelia incarnata* and *S. propinqua* in the moorland these were not visited (Chapters 4 and 5). More specific relationships such as this are likely to come to light as further information becomes available.

In some epacrids, differences in visitor profiles occur in the absence of major differences in floral morphology and often vary within a genus. For instance, while the potential pollinators of the brush-like flowers of *Richea* overlap, each species has its own combination of at least two of bird, fly, bee, beetle, and wind pollination systems (Chapter 5). Also *Leucopogon* taxa have been reported to have only insect visitors but the visitors vary between congeners and between sites. Hingston and McQuillan (2000) suggest that *Leucopogon collinus* supports large numbers of bees and flies, while *L. ericoides, L. parvifolia* and *L. virgatus* are predominantly bee visited. To add further complexity, *L. collinus* varies in floral visitor profile between sites – sometimes bees are almost exclusive visitors, sometimes flies, and sometimes an equal diversity of both (Hingston and McQuillan 2000). Thus, while the information collated here gives an overview of the probable trends in epacrid pollination it is almost certain that broader sampling of specific species across different environments would refine the pollination systems outlined.

In contrast, some genera include vastly different floral morphologies that are associated with different pollination systems. For example, *Trochocarpa* includes taxa with red and hairy flowers visited by birds and taxa with white open cup-shaped flowers visited by insects. Variability is also apparent within some *Epacris*. *Epacris impressa* is floristically variable with a variable visitor profile (Fig. 7-2) and has some populations with polymorphic corolla colours and others with monomorphic corolla colours: white, pink or scarlet. It has so much variation that Stace and Fripp (1977) described the following races: scarlet corollas 15-19 mm long and creamy white anthers; pink corollas 12-19 mm long with creamy white anthers; white corolla 9-12 mm long and purple-red anthers; *E. grandiflora* which usually has

polymorphic pink and white-flowered populations. This suggests a general association between longer corollas in the pink and red-flowered races and shorter corollas in the white-flowered race and a potential bias towards bird and insect pollinators respectively (Stace and Fripp, 1977). In support of this theory, Hingston and McQuillan (2000) found uniformly pink-flowered populations to be regularly visited by birds but not by insects, but they also found that mixed white and pink populations that exhibited shorter corolla tubes than the purely pink populations were visited by both. In the broader Ericaceae, morphological variation and colour polymorphism are seen in *Erica* taxa that have large altitudinal ranges or occur at low elevations, have long flowering periods or bloom in autumn, and are pollinated by birds (Rebelo and Siegfried, 1985). Although, Rebelo and Siegfried (1985) also suggest that the high incidence of colour polymorphism in some *Erica* species may be a result of the same unknown factors which influenced speciation and the high endemicity of the genus.

To explain the floral diversity of epacrids and how changes in floral morphology may reflect changes in pollinator(s) it is essential to consider the malleability of the floral features. Overall flower shape is under genetic constraint (Whitney and Glover, 2007), hence the actinomorphic bauplan of epacrids. Although actinomorphic flowers represent the default floral form, there are numerous examples of changes to zygomorphic flowers and some changes in the reverse direction (Endress, 2001; Whitney and Glover, 2007). Furthermore, floral symmetry may be disrupted by a mutation to any one of the several genes that control it (Whitney and Glover, 2007). Thus, it is potentially interesting that this speciose subfamily has not undergone any changes in basic architecture. However, changes in shape can also be effected by altering the size of one or more organs – consider the differences between the large flowers of *Styphelia* with their protruding reproductive organs and the small flowers of *Leucopogon* with their reproductive organs hidden inside the corolla. Also consider the within species variability of *Epacris impressa* previously described. As flower size tends not to be variable within a species (Whitney and Glover, 2007) contrary plants such as *E. impressa* are particularly worthy of further scrutiny. Even though petal shape and curvature are strongly constrained (Whitney and Glover, 2007) there are other ways of altering the appearance of the flower. The colour of a flower is relatively plastic (Whitney and Glover, 2007) and thus may provide a good basis for assessing associations with pollinator-type. While a relationship between red flowers and bird pollination has been established, there are other flower colours that occur in independent parts of the tree, including yellow and green. Based on the findings

here, fruitful areas of future research into epacrid-pollinator associations are most likely to be found where flowers have undergone alterations to shape or colour at the tips of the evolutionary tree.

In conclusion, using customised pollination syndromes to infer pollinators where they were unknown provided a more complete hypothesis for the pollination ecology and evolution of epacrids. Exploring the evolutionary relationships between floral traits and pollinator profiles has led to detection of convergent evolution of floral traits and their likely association to particular pollinator groups. Classification models were relatively successful at predicting, in particular, bird-visited epacrids (86%). By comparison, the predictive utility of the traditional pollination syndromes, when tested globally, was unsuccessful in more than half the plant taxa assessed (Ollerton et al. 2009). While the accuracy of pollination syndromes as universal predictors remains in question, using a subset of known potential pollinators to inform the prediction process provides the benefit of a known level of certainty for a prediction. In epacrids, the tangible differences in pollination syndromes of birds versus insects provide great utility in understanding the evolution of floral features in the subfamily. The adaptations of floral traits to pollinators appear to have played a major role in the morphological diversification of the epacrids.

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7.1 Appendices

Appendix 7-1 Field observations – site summary

With the purpose of supplementing the pollination studies available from the literature, I made opportunistic field observations in southern Australia between 2007 and 2009, on 14 taxa from *Dracophyllum*, *Epacris*, *Leptecophylla, Leucopogon*, *Lissanthe*, *Monotoca* and *Trochocarpa* (Table A). Observations were made between 10.00 and 16.00 hours on fine clear days with little wind (< 3 m per sec) during peak flowering times. Flowers with available nectar and pollen were chosen for observation in person and by video camera (Panasonic Digital Video Camera, model no. NV-GS70, 1.7 MP, 500x digital zoom and /or JVC Digital Video Camera, model no. GZ-MG465, 1.07 MP, 32x optical zoom) on a tripod.

Samples of the foraging insects were collected by netting or capturing directly into a plastic screw-top container wetted with ethanol. Insects were killed and stored in screw-top vials with 70% ethanol. Bees were identified to genus under a dissecting microscope using the key of Michener (1965) and the Hingston bee collection (housed at the School of Geography and Environmental Studies Laboratory, UTAS) which holds specimens determined by Dr. Ken Walker (National Museum of Victoria). Flies were identified using Colless and McAlpine (1991) and butterflies using Braby (2004). Other invertebrates were identified using Zborowski and Storey (2003), Daley (2007) and Shattuck (1999). Bird visitors were identified in the field with binoculars or from close-up photographs and video imagery as they visited flowers using Pizzey and Knight (1997). Voucher specimens of potential pollinators collected during surveys are held at the School of Geography and Environmental Studies Laboratory (University of Tasmania).

Table A. Epacrid site summary

Location	Coordinates	Species
Mt. Field National Park	42°39'29"S 146°31'04"E	*Dracophyllum minimum*
	42°39'29"S 146°31'04"E	*Epacris serpyllifolia*
	42°40'68"S 146°38'22"E	*Trochocarpa thymifolia*
	42°39'44"S 146°38'57"E	*Monotoca empetrifolia*
Tasmanian Wilderness World Heritage Area	42°49'55"S 146°23'06"E	*Trochocarpa gunnii*
Echo Sugarloaf State Reserve	43°14'43"S 147°07'52"E	*Lissanthe strigosa*
Randalls Bay Conservation Area	43°14'41"S 147°07'55"E	*Epacris impressa*
	43°14'41"S 147°07'55"E	*Leucopogon ericoides*
Egg and Bacon Bay Road	43°14'45"S 147°07'04"E	*Epacris lanuginosa*
Huon Road	42°54'12"S 147°16'50"E	*Epacris impressa*
Ridgeway Park	42°55'09"S 147°17'01"E	*Leucopogon virgatus*
	42°55'09"S 147°17'01"E	*Leucopogon collinus*
Remarkable Cave State Reserve	43°11'12"S 147°50'48"E	*Epacris marginata*
	43°11'12"S 147°50'48"E	*Leucopogon parviflorus*
Cape Raoul State Reserve	43°13'05"S 147°47'05"E	*Leptecophylla juniperina* subsp. *juniperina*

Appendix 7-2 Potential pollinators of epacrids (sorted by Tribe, Plant species, Common name (of potential pollinator); bold = original contributions from this thesis; * = used in RF analyses. Note that plant species names are as they appear in original studies) - see over page

	Code	Tribe	Plant species	Potential pollinator	Common name	Scientific name	Source
*	Ag	Cosmelieae	*Andersonia grandiflora*	Bird	New-Holland Honeyeater	*Phylidonyris novaehollandiae*	Keighery (1996)
*	Ah	Cosmelieae	*Andersonia heterophylla*	Insect	Bee	*Leioproctus macmillani*	Houston (2000)
*	Am	Cosmelieae	*Andersonia micrantha*	Insect	Fly	Unknown	Keighery (1996)
*	Ase	Cosmelieae	*Andersonia setifolia*	Bird	White-Cheeked Honeyeater	*Phylidonyris higra*	Keighery (1996)
*	Asp	Cosmelieae	*Andersonia sprengeloides*	Insect	Butterfly	Unknown	Keighery (1996)
*	Asp	Cosmelieae	*Andersonia sprengeloides*	Insect	Moth	Unknown	Keighery (1996)
*	Cru	Cosmelieae	*Cosmelia rubra*	Bird	Brown-Headed Honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Cru	Cosmelieae	*Cosmelia rubra*	Bird	New Holland Honeyeater	*Phylidonyris novaehollandiae*	Keighery (1996)
*	**Si**	**Cosmelieae**	***Spengelia incarnata***	**Insect**	**Bee**	***Euryglossa* sp.**	**Johnson and McQuillan (2011); Chapter 4**
*	**Si**	**Cosmelieae**	***Spengelia incarnata***	**Insect**	**Bee**	***Exoneura* sp.**	**Johnson and McQuillan (2011); Chapter 4**
*	Si	Cosmelieae	*Spengelia incarnata*	Insect	Bee	*Homalictus niveifrons/megastigmus*	Hingston (1999)
*	**Si**	**Cosmelieae**	***Spengelia incarnata***	**Insect**	**Bee**	***Lasioglossum* (*Chilalictus*) sp.**	**Johnson and McQuillan (2011); Chapter 4**
*	Si	Cosmelieae	*Spengelia incarnata*	Insect	Bee	*Lasioglossum* (*Ctenonomia*) sp.	Hingston (1999)
*	**Si**	**Cosmelieae**	***Spengelia incarnata***	**Insect**	**Bee**	***Lasioglossum* (*Parasphecodes*) sp.**	**Johnson and McQuillan (2011); Chapter 4**
*	Si	Cosmelieae	*Spengelia incarnata*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Si	Cosmelieae	*Spengelia incarnata*	Insect	Bee	*Leioproctus* (*Leioproctus*) sp.	Hingston (1999)
*	Si	Cosmelieae	*Spengelia incarnata*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	**Sp**	**Cosmelieae**	***Spengelia propinqua***	**Insect**	**Bee**	***Lasioglossum* (*Chilalictus*) sp.**	**Johnson and McQuillan (2011); Chapter 4**
*	**Sp**	**Cosmelieae**	***Spengelia propinqua***	**Insect**	**Bee**	***Lasioglossum* (*Parasphecodes*) sp.**	**Johnson and McQuillan (2011); Chapter 4**
*	**Sp**	**Cosmelieae**	***Spengelia propinqua***	**Insect**	**Fly**	**Syriphidae**	**Johnson and McQuillan (2011); Chapter 4**
	Sp	**Cosmelieae**	***Spengelia propinqua***	**Insect**	**Honeybee**	***Apis mellifera***	**Johnson and McQuillan (2011); Chapter 4**
*	**Ec**	**Epacrideae**	***Epacris corymbifolia***	**Insect**	**Butterfly**	***Graphium macleayanus***	**Johnson and McQuillan (2011); Chapter 4**

*	**Ec**	**Epacrideae**	***Epacris corymbifolia***	**Insect**	**Butterfly**	***Graphium macleayanus***	**Johnson and McQuillan (2011); Chapter 4**
*	Ei	Epacrideae	*Epacris impressa*	Insect	Bee	*Exoneura* sp.	Hingston (1999)
*	Ei	Epacrideae	*Epacris impressa*	Insect	Bee	*Homalictus niveifrons/megastigmus*	Hingston (1999)
*	Ei	Epacrideae	*Epacris impressa*	Insect	Bee	*Lasioglossum* (*Chilalictus*) sp.	Bernhardt (1986)
*	Ei	Epacrideae	*Epacris impressa*	Insect	Bee	*Lasioglossum* (*Ctenonomia*) sp.	Hingston (1999)
*	Ei	Epacrideae	*Epacris impressa*	Insect	Bee	*Leioproctus* (*Leioproctus*) sp.	Hingston (1999)
*	Ei	Epacrideae	*Epacris impressa*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Insect**	**Bee**	**Unknown**	**KAJ (Nov 2009)**
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Insect**	**Beetle**	***Chauliognathus* sp.**	**KAJ (Sep 2007)**
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Insect**	**Beetle**	**Unknown**	**KAJ (Sep 2007)**
	Ei	**Epacrideae**	***Epacris impressa***	**Insect**	**Bumblebee**	***Bombus terrestris***	**KAJ (Oct 2009)**
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Insect**	**Bumblebee**	***Bombus terrestris***	**KAJ (Sep 2008)**
*	Ei	Epacrideae	*Epacris impressa*	Insect	Butterfly	Unknown	Hingston and McQuillan (2000)
*	Ei	Epacrideae	*Epacris impressa*	Bird	Crescent Honeyeater	*Phylidonyris pyrrhoptera*	Paton and Ford (1977)
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Bird**	**Eastern spinebill**	***Acanthorhynchus tenuirostris***	**KAJ (May 2007)**
*	Ei	Epacrideae	*Epacris impressa*	Bird	Eastern spinebill	*Acanthorhynchus tenuirostris*	Paton and Ford (1977)
*	Ei	Epacrideae	*Epacris impressa*	Bird	Eastern spinebill	*Acanthorhynchus tenuirostris*	Thomas (1980)
*	Ei	Epacrideae	*Epacris impressa*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ (Oct 2009)**
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ (Sep 2008)**
*	Ei	Epacrideae	*Epacris impressa*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Ford et al.(1979)
*	Ei	Epacrideae	*Epacris impressa*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Paton and Ford (1977)
*	Ei	Epacrideae	*Epacris impressa*	Bird	Unknown	Unknown	Hingston and McQuillan (2000)
*	Ei	Epacrideae	*Epacris impressa*	Bird	White-plumed honeyeater	*Lichenostomus penicillatus*	Paton and Ford (1977)
*	**Ei**	**Epacrideae**	***Epacris impressa***	**Bird**	**Yellow-throated honeyeater**	***Lichenostomus flavicollis***	**KAJ (Apr 2011)**
*	El	Epacrideae	*Epacris lanuginosa*	Insect	Bee	*Exoneura* sp.	Hingston (1999)
*	**El**	**Epacrideae**	***Epacris lanuginosa***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ (Oct 2009)**
*	El	Epacrideae	*Epacris lanuginosa*	Insect	Bee	*Lasioglossum* (*Ctenonomia*)	Hingston (1999)
*	El	Epacrideae	*Epacris lanuginosa*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	El	Epacrideae	*Epacris lanuginosa*	Insect	Butterfly	Unknown	Hingston and McQuillan (2000)
*	El	Epacrideae	*Epacris lanuginosa*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
	El	**Epacrideae**	***Epacris lanuginosa***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ (Oct 2009)**

*	**Em**	**Epacrideae**	***Epacris marginata***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ (Oct 2007)**
*	**Em**	**Epacrideae**	***Epacris marginata***	**Insect**	**Bee**	***Lasioglossum* sp.**	**KAJ (Oct 2007)**
	Em	**Epacrideae**	***Epacris marginata***	**Insect**	**Bumblebee**	***Bombus terrestris***	**Johnson *et al.* (2010); Chapter 2**
	Em	**Epacrideae**	***Epacris marginata***	**Insect**	**Bumblebee**	***Bombus terrestris***	**KAJ (Oct 2007)**
*	**Em**	**Epacrideae**	***Epacris marginata***	**Insect**	**Fly**	**Stratiomyidae**	**KAJ (Oct 2007)**
*	**Em**	**Epacrideae**	***Epacris marginata***	**Insect**	**Fly**	**Tachinidae**	**KAJ (Oct 2007)**
	Em	**Epacrideae**	***Epacris marginata***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ (Oct 2007)**
*	Ep	Epacrideae	*Epacris paludosa*	Insect	Butterfly	Lycaenidae (blues)	Green and Osborne (1994)
*	Epe	Epacrideae	*Epacris petrophila*	Insect	Butterfly	Lycaenidae (blues)	Green and Osborne (1994)
*	**Es**	**Epacrideae**	***Epacris serpyllifolia***	**Insect**	**Beetle**	***Chauliognathus* sp.**	**KAJ (Jan 2009)**
*	**Es**	**Epacrideae**	***Epacris serpyllifolia***	**Insect**	**Beetle**	**Elateridae**	**KAJ (Jan 2009)**
*	**Es**	**Epacrideae**	***Epacris serpyllifolia***	**Insect**	**Butterfly**	***Graphium macleayanus***	**KAJ (Jan 2009)**
*	Es	Epacrideae	*Epacris serpyllifolia*	Insect	Butterfly	Lycaenidae (blues)	Green and Osborne (1994)
*	Es	Epacrideae	*Epacris serpyllifolia*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	**Es**	**Epacrideae**	***Epacris serpyllifolia***	**Insect**	**Fly**	**Unknown**	**KAJ (Jan 2009)**
*	Est	Epacrideae	*Epacris stuartii*	Insect	Fly	*Calliphora hilli*	Keith and Ilowski (1999)
*	Est	Epacrideae	*Epacris stuartii*	Insect	Fly	*Calliphora* sp.	Keith and Ilowski (1999)
*	Lsp	Epacrideae	*Leucopogon* spp.	Insect	Bee	*Lasioglossum* (Chilalictus) sp.	Bernhardt (1986)
*	Lsp	Epacrideae	*Leucopogon* spp.	Insect	Bee	*Leioproctus* sp.	Bernhardt (1986)
*	Lci	Epacrideae	*Lysinema ciliatum*	Insect	Moth	Unknown	Keighery (1996)
*	Lel	Epacrideae	*Lysinema elegans*	Insect	Moth	Unknown	Keighery (1996)
*	Lf	Epacrideae	*Lysinema fimbriatum*	Insect	Butterfly	Unknown	Keighery (1996)
*	Np	Oligarrheneae	*Needhamiella pumilio*	Insect	Moth	*Pollanisus viridipulverulenta*	Keighery (1996)
	Pc	**Prionteae**	***Prionotes cerinthoides***	**Insect**	**Bumblebee**	***Bombus terrestris***	**Johnson *et al.* (2010); Chapter 2**
*	**Pc**	**Prionteae**	***Prionotes cerinthoides***	**Bird**	**Crescent honeyeater**	***Phylidonyris pyrrhoptera***	**Johnson *et al.* (2010); Chapter 2**
*	**Pc**	**Prionteae**	***Prionotes cerinthoides***	**Bird**	**Eastern spinebill**	***Acanthorhynchus tenuirostris***	**Johnson *et al.* (2010); Chapter 2**
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	"*Eucymatoge*" *gobiata*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	"*Hydriomena*" *deltoidata*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	"*Hydriomena*" *rixata*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	"*Leucania*" *toroneura*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Declana junctilinea*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Eudonia sabulosella*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Graphania sequens*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Orocrambus flexuosellus*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Pasiphila biolineolata*	Primack (1983)

*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Pasiphila* sp. near *dryas*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Rictonis comma*	Primack (1983)
*	Da	Richeeae	*Dracophyllum acerosum*	Insect	Moth	*Scoparia submarginalis*	Primack (1983)
*	Dac	Richeeae	*Dracophyllum acicularifolium*	Insect	Beetle	*Dasytes* sp.	Heine (1935)
*	Dl	Richeeae	*Dracophyllum longifolium*	Inset	Moth	*Xanthorhoe umbrosa*	Thomson (1926)
*	Dl	Richeeae	*Dracophyllum longifolium*	Bird	Unknown	Unknown	Thomson (1926)
*	Dm	Richeeae	*Dracophyllum minimum*	Insect	Bee	*Apis melifera*	Corbett (1995)
*	Dm	Richeeae	*Dracophyllum minimum*	Insect	Beetle	*Chauliognathus* sp.	Corbett (1995)
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Beetle**	***Chauliognathus tricolor***	**KAJ Chapter 5**
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Beetle**	**Elateridae**	**KAJ Chapter 5**
*	Dm	Richeeae	*Dracophyllum minimum*	Insect	Beetle	*Hypatallus* sp.	Corbett (1995)
	Dm	Richeeae	*Dracophyllum minimum*	Insect	Bug	Lygaeidae	Corbett (1995)
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Butterfly**	***Graphium macleayanus***	**KAJ Chapter 5**
	Dm	Richeeae	*Dracophyllum minimum*	Insect	Fly	*Calliphora* sp.	Corbett (1995)
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Fly**	**Calliphoridae**	**KAJ Chapter 5**
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Fly**	**Pelecorhynchidae**	**KAJ Chapter 5**
	Dm	Richeeae	*Dracophyllum minimum*	Insect	Fly	Tabanidae	Corbett (1995)
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Fly**	**Tabinidae**	**KAJ Chapter 5**
*	**Dm**	**Richeeae**	***Dracophyllum minimum***	**Insect**	**Fly**	**Tachinidae**	**KAJ Chapter 5**
	Dm	Richeeae	*Dracophyllum minimum*	Insect	Grasshopper	*Russalpia albertisi*	Corbett (1995)
	Dm	Richeeae	*Dracophyllum minimum*	Insect	Beetle	*Sessinia sublineata*	Corbett (1995)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Beetle	Unknown	Primack (1983)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Fly	*Avibrissia isolata*	Primack (1983)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Fly	*Neotachina* sp.	Primack (1983)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Fly	*Veluta albicineta*	Primack (1983)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Moth	*Dasyuris anceps*	Primack (1983)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Moth	*Notoreas anthracias*	Primack (1983)
*	Dp	Richeeae	*Dracophyllum pronum*	Insect	Moth	*Plagiomya turbidum*	Primack (1983)
*	Dra	Richeeae	*Dracophyllum ramosum*	Insect	Beetle	Unknown	Kato and Kawakita (2004)
*	Dra	Richeeae	*Dracophyllum ramosum*	Insect	Moth	Unknown	Kato and Kawakita (2004)
*	Dr	Richeeae	*Dracophyllum rosmarinifolium*	Insect	Beetle	*Platyomida caudata*	Heine (1935)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	"*Eucymatoge*" *gobiata*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	"*Hydriomena*" *deltoidata*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	"*Hydriomena*" *rixata*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	"*Leucania*" *toroneura*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Declana junctilinea*	Primack (1983)

*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Eudonia sabulosella*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Graphania sequens*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Orocrambus flexuosellus*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Pasiphila biolineolata*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Pasiphila* sp. near *dryas*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Rictonis comma*	Primack (1983)
*	Du	Richeeae	*Dracophyllum uniflorum*	Insect	Moth	*Scoparia submarginalis*	Primack (1983)
	Dv	Richeeae	*Dracophyllum verticillatum*	Insect	Honeybee	*Apis mellifera*	Kato and Kawakita (2004)
*	**Ra**	**Richeeae**	***Richea acerosa***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ Chapter 5**
*	**Ra**	**Richeeae**	***Richea acerosa***	**Insect**	**Bee**	***Lasioglossum parasephecodes***	**KAJ Chapter 5**
*	**Ra**	**Richeeae**	***Richea acerosa***	**Insect**	**Fly**	**Calliphoridae**	**KAJ Chapter 5**
*	**Ra**	**Richeeae**	***Richea acerosa***	**Insect**	**Fly**	**Syrphidae**	**KAJ Chapter 5**
*	**Ra**	**Richeeae**	***Richea acerosa***	**Insect**	**Fly**	**Tabinidae**	**KAJ Chapter 5**
	Ra	**Richeeae**	***Richea acerosa***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ Chapter 5**
*	Rc	Richeeae	*Richea contentinalis*	Insect	Bee	*Exoneura*	Green and Osborne (1994)
*	Rc	Richeeae	*Richea contentinalis*	Insect	Bee	*Hylaeus*	Green and Osborne (1994)
*	Rc	Richeeae	*Richea contentinalis*	Insect	Bee	*Lasioglossum*	Green and Osborne (1994)
*	Rc	Richeeae	*Richea contentinalis*	Insect	Bee	*Leioproctus*	Green and Osborne (1994)
*	Rc	Richeeae	*Richea contentinalis*	Insect	Fly	*Calliphora*	Green and Osborne (1994)
*	Rc	Richeeae	*Richea contentinalis*	Insect	Fly	*Scaptia*	Green and Osborne (1994)
*	**Rd**	**Richeeae**	***Richea dracophylla***	**Insect**	**Bee**	***Leioproctus* sp.**	**KAJ Chapter 5**
*	**Rd**	**Richeeae**	***Richea dracophylla***	**Bird**	**Crescent honeyeater**	***Phylidonyris pyrrhoptera***	**KAJ Chapter 5**
*	**Rd**	**Richeeae**	***Richea dracophylla***	**Insect**	**Fly**	**Syrphidae**	**KAJ Chapter 5**
*	**Rd**	**Richeeae**	***Richea dracophylla***	**Insect**	**Wasp**	**Ichneumonidae**	**KAJ Chapter 5**
*	**Rd**	**Richeeae**	***Richea dracophylla***	**Bird**	**Yellow wattlebird**	***Anthocaera paradoxa***	**KAJ Chapter 5**
*	**Rm**	**Richeeae**	***Richea milliganii***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ Chapter 5**
*	**Rm**	**Richeeae**	***Richea milliganii***	**Insect**	**Bee**	***Lasioglossum parasephecodes***	**KAJ Chapter 5**
*	**Rm**	**Richeeae**	***Richea milliganii***	**Insect**	**Fly**	**Syrphidae**	**KAJ Chapter 5**
	Rm	**Richeeae**	***Richea milliganii***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ Chapter 5**
*	**Rm**	**Richeeae**	***Richea milliganii***	**Insect**	**Wasp**	***Heteropelma* sp.**	**KAJ Chapter 5**
*	**Rp**	**Richeeae**	***Richea pandanifolia***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ Chapter 5**
*	Rp	Richeeae	*Richea pandanifolia*	Insect	Beetle	Aleocharinae	P. McQuillan (University of Tasmania) pers. comm. 2007
*	**Rp**	**Richeeae**	***Richea pandanifolia***	**Bird**	**Crescent honeyeater**	***Phylidonyris pyrrhoptera***	**KAJ Chapter 5**

*	**Rp**	**Richeeae**	***Richea pandanifolia***	**Insect**	**Fly**	**Syrphidae**	**KAJ Chapter 5**
*	**Rp**	**Richeeae**	***Richea pandanifolia***	**Insect**	**Fly**	**Tabinidae**	**KAJ Chapter 5**
*	**Rp**	**Richeeae**	***Richea pandanifolia***	**Bird**	**Yellow-throated honeyeater**	***Lichenostomus flavicollis***	**KAJ Chapter 5**
*	Rp	Richeeae	*Richea pandanifolia*	Bird	Unknown	Unknown	Hingston and McQuillan (2000)
*	**Rpr**	**Richeeae**	***Richea procera***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ Chapter 5**
*	**Rpr**	**Richeeae**	***Richea procera***	**Insect**	**Fly**	**Calliphoridae**	**KAJ Chapter 5**
	Rpr	**Richeeae**	***Richea procera***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ Chapter 5**
*	Rpr	Richeeae	*Richea procera*	Wind	Na	Na	Ladd (2006)
*	**Rs**	**Richeeae**	***Richea scoparia***	**Insect**	**Beetle**	***Chauliognathus tricolor***	**KAJ Chapter 5**
	Rs	Richeeae	*Richea scoparia*	Insect	Bumblebee	*Bombus terrestris*	Olsson *et al.* (2000)
*	**Rs**	**Richeeae**	***Richea scoparia***	**Insect**	**Fly**	**Calliphoridae**	**KAJ Chapter 5**
*	**Rs**	**Richeeae**	***Richea scoparia***	**Insect**	**Fly**	**Tachinidae**	**KAJ Chapter 5**
*	Rs	Richeeae	*Richea scoparia*	Insect	Fly	Unknown	Olsson *et al.* (2000)
*	**Rs**	**Richeeae**	***Richea scoparia***	**Lizard**	**Snow skink**	***Niveoscincus* sp.**	**KAJ Chapter 5**
*	Rs	Richeeae	*Richea scoparia*	Lizard	Snow skink	Unknown	Olsson *et al.* (2000)
*	Rs	Richeeae	*Richea scoparia*	Insect	Wasp	Unknown	Olsson *et al.* (2000)
*	**Rsp**	**Richeeae**	***Richea sprengelioides***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ Chapter 5**
*	**Rsp**	**Richeeae**	***Richea sprengelioides***	**Insect**	**Beetle**	**Elateridae**	**KAJ Chapter 5**
*	**Rsp**	**Richeeae**	***Richea sprengelioides***	**Insect**	**Fly**	**Heleomyzidae**	**KAJ Chapter 5**
*	**Rsp**	**Richeeae**	***Richea sprengelioides***	**Insect**	**Fly**	**Syrphidae**	**KAJ Chapter 5**
*	Rsp	Richeeae	*Richea sprengelioides*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
	Rsp	**Richeeae**	***Richea sprengelioides***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ Chapter 5**
*	Rsp	Richeeae	*Richea sprengelioides*	Wind	Na	Na	Ladd (2006)
*	Rv	Richeeae	*Richea victoriana*	Wind	Na	Na	Ladd (2006)
	Ssp	Richeeae	*Sphenotoma* sp.	Insect	Bee	*Amegilla pulchra*	Keighery (1996)
	Ssp	Richeeae	*Sphenotoma* sp.	Insect	Moth	Unknown	Keighery (1996)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Anonchomyrma itinerans*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Camponotus fergusoni*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Camponotus sponsorum*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Camponotus terebrans*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Camponotus* undescribed sp. B	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Crematogaster* sp.	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Crematogaster* sp. A (*queenslandica* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Froggattella kirbii*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Iridomyrmex chaseri*	Schneemilch et al (2011)

	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Iridomyrmex* sp. (*rufoniger* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Iridomyrmex* sp. J (*myjobergi* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Monomorium* sp. (*flavipes* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Monomorium* sp. (*longiceps* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Notoncus enormis*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Notoncus hickmani*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Notoncus rotundiceps*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Notoncus* sp.	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Notoncus* sp. (*hickmani* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Polyrhachis* sp. (*sidnica* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Rhytidoponera metallica*	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Tapinoma* sp. A (*minutum* gp.)	Schneemilch et al (2011)
	Aa	Styphelieae	*Acrotriche affinis*	Insect	Ant	*Tapinoma* sp. C (*minutum* gp.)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus consobrinus*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus donnellani*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus frivola*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus guidea*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus lownei*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus* sp.	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Camponotus terebrans*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Crematogaster dispar*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Froggattella kirbii*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Iridomyrmex* (nr *bicknelli*)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Iridomyrmex fusciventris*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Iridomyrmex prismatus*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Iridomyrmex* sp. (nr *suchieri*)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Iridomyrmex* sp. (*rufoniger* gp.)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Melophorus* sp. (*turneri* gp.)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Myrmecia dichospila*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Myrmecorhynchus emeryi*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Notoncus hickmani*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Notoncus rotundiceps*	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Notoncus* sp.	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Notoncus* sp. (*hickmani* gp.)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Ochetellus* sp. (*glaber* gp.)	Schneemilch et al (2011)
	Ac	Styphelieae	*Acrotriche cordata*	Insect	Ant	*Tapinoma* sp.	Schneemilch et al (2011)

*	Ac	Styphelieae	*Acrotriche cordata*	Insect	Fly	Muscid Flies	Keighery (1996)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus claripes*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus consobrinus*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus guidea*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus judithmorrisae*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus lownei*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus oetkeri*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus piliventris*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus rudis*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Camponotus* sp.	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Crematogaster dispar*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Crematogaster laeviceps*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Crematogaster* sp. (*queenslandica* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	Dolichoderus sp. (scabridus gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Iridomyrmex* (nr *bicknelli*)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Iridomyrmex chasei*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Iridomyrmex dromus*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Iridomyrmex purpureus*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Iridomyrmex* sp. A (*splendens* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Melophorus* sp. (*aeneovirans* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Monomorium kilianii*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Monomorium* sp. (*sordidum* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Monomorium* sp. (*leave* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Myrmecia dichospila*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Myrmecia forceps*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Myrmecia nigroscarpa*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Myrmecia pilosula*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Myrmecia pyriformis*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Ochetellus* sp. (*glaber* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Orectognathus clarki*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Pheidole* sp. (*ampla* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Podomyrma sp. (rugosa gp.)*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Polyrhachis* sp. (*sidnica* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Polyrhachis* sp. nr *phryne*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Prolasius* sp.	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Rhytidoponera metallica*	Schneemilch et al (2011)

	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Stigmacros aemula*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Stigmacros* sp. (*rufa* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Stigmacros* sp. nr *barretti*	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Tapinoma* sp. (*minutum* gp.)	Schneemilch et al (2011)
	Ad	Styphelieae	*Acrotriche depressa*	Insect	Ant	*Technomyrmex jocusus*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Anonychomyrma* sp. *(nitidiceps* gp.*)*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Dolichoderus* sp. (*scabridus* gp.)	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Iridomyrmex purpureus*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Monomorium kilianii*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Myrmecia sp. (nr pyriformis)*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Notoncus enormis*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Notoncus spinisquamus*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Ochetellus* sp. (*glaber* gp.)	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Prolasius* sp.	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Prolasius* sp. (*nitidissimus* gp.)	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Rhytidoponera metallica*	Schneemilch et al (2011)
	Af	Styphelieae	*Acrotriche fasciculiflora*	Insect	Ant	*Technomyrmex jocusus*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus capito ebinithorax*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus claripes*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus consobrinus*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus fergusoni*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus guidea*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus rudis*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Camponotus terebrans*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Crematogaster laeviceps	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Crematogaster sp. (queenslandica gp.)	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Iridomyrmex chasei	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Iridomyrmex purpureus*	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Melophorus froggatti	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Melophorus sp.	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Meranoplus similis	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Notoncus hickmani	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Notoncus rotundiceps	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	Prolasius sp.	Schneemilch et al (2011)
	Ap	Styphelieae	*Acrotriche patula*	Insect	Ant	*Tapinoma* sp. *(minutum* gp.*)*	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Camponotus gaseri	Schneemilch et al (2011)

	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Crematogaster laeviceps	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Dolichoderus sp. (scabridus gp.)	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Epopostruma frosti	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Iridomyrmex purpureus	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Monomorium kilianii	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Monomorium sp. (sordidum gp.)	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Notoncus hickmani	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	*Notoncus* sp. (*hickmani* gp.)	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Ochetellus sp. (glaber gp.)	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Prolasius sp.	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Rhytidoponera metallica	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Insect	Ant	Tapinoma sp. (minutum gp.)	Schneemilch et al (2011)
	As	Styphelieae	*Acrotriche serrulata*	Mammal	Antechinus	*Antechinus stuartii*	Fletcher (1977)
	As	**Styphelieae**	***Acrotriche serrulata***	**Mammal**	**Black Rat**	***Rattus rattus***	**Johnson et al. (2011); Chapter 3**
	As	**Styphelieae**	***Acrotriche serrulata***	**Mammal**	**Common brushtail possum**	***Trichosurus vulpecula***	**Johnson et al. (2011); Chapter 3**
*	Aci	Styphelieae	*Astroloma ciliatum*	Bird	Western spinebill	*Acanthorhynchus superciliosus*	Keighery (1996)
*	Acom	Styphelieae	*Astroloma compactum*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Keighery (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	bird	Adelaide rosella	*Platycercus elegans*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Black chinned honeyeater	*Melithreptus gularis*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Crescent honeyeater	*Phylidonyris pyrrhoptera*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Crescent honeyeater	*Phylidonyris pyrrhoptera*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Eastern spinebill	*Acanthorhynchus tenuirostris*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Eastern spinebill	*Acanthorhynchus tenuirostris*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Little wattlebird	*Anthochaera chrysoptera*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Ford et al. (1979)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Purple crowned lorikeet	*Glossopsitta porphyrocephala*	Paton and Ford (1977)

*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Red wattlebird	*Anthochaera carunculata*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Red wattlebird	*Anthochaera carunculata*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Silvereye	*Zosterops lateralis*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Silvereye	*Zosterops lateralis*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Striated thornbill	*Acanthiza lineata*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Tawny crowned honeyeater	*Phylidonyris melanops*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	White naped honeyeater	*Melithreptus lunatus*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	White naped honeyeater	*Melithreptus lunatus*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	White plumed honeyeater	*Lichenostomus penicillatus*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	White plumed honeyeater	*Lichenostomus penicillatus*	Wheal (1996)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Yellow faced honeyeater	*Lichenstomus chrysops*	Paton and Ford (1977)
*	Aco	Styphelieae	*Astroloma conostephioides*	Bird	Yellow faced honeyeater	*Lichenstomus chrysops*	Wheal (1996)
*	Adr	Styphelieae	*Astroloma drummondii*	Bird	White cheeked honeyeater	*Phylidonyris higra*	Keighery (1996)
*	Ae	Styphelieae	*Astroloma epacridis*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Afo	Styphelieae	*Astroloma foliosum*	Bird	Western spinebill	*Acanthorhynchus superciliosus*	Keighery (1996)
*	Agl	Styphelieae	*Astroloma glaucescens*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Am	Styphelieae	*Astroloma macrocalyx*	Bird	Western spinebill	*Acanthorhynchus superciliosus*	Keighery (1996)
*	Ami	Styphelieae	*Astroloma microcalyx*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Keighery (1996)
*	Apa	Styphelieae	*Astroloma pallidum*	Bird	Western spinebill	*Acanthorhynchus superciliosus*	Keighery (1996)
*	Apr	Styphelieae	*Astroloma prostratum*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Keighery (1996)
*	Aser	Styphelieae	*Astroloma serratifolium*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Ase	Styphelieae	*Astroloma serratifolium*	Bird	Tawny-Crowned Honeyeater	*Phylidonyris melanops*	Keighery (1996)
*	Ax	Styphelieae	*Astroloma xerophyllum*	Insect	Bee	*Leioproctus macmillani*	Houston (2000)

	Bc	Styphelieae	*Brachyloma concolor*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Bd	Styphelieae	*Brachyloma daphnoides*	Insect	Beetle	*Metriorrhynchus rhipidius*	Hawkeswood (2002)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Crescent Honeyeater	*Phylidonyris pyrrhoptera*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	bird	Eastern spinebill	*Acanthorhynchus tenuirostris*	Celebrezze and Paton (2004)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Eastern Spinebill	*Acanthorhynchus tenuirostris*	Paton and Ford (1977)
	Be	Styphelieae	*Brachyloma ericoides*	insect	Honeybee	*Apis mellifera*	Celebrezze and Paton (2004)
*	Be	Styphelieae	*Brachyloma ericoides*	bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Celebrezze and Paton (2004)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	New Holland honeyeater	*Phylidonyris novaehollandiae*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Purple gaped Honeyeater	*Lichenostomus cratitius*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Red wattlebird	*Anthochaera carunculata*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	bird	Silvereye	*Zosterops lateralis*	Celebrezze and Paton (2004)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Silvereye	*Zosterops lateralis*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	bird	Tawny crowned honeyeater	*Phylidonyris melanops*	Celebrezze and Paton (2004)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Tawny crowned honeyeater	*Phylidonyris melanops*	Paton and Ford (1977)
*	Be	Styphelieae	*Brachyloma ericoides*	Bird	Yellow throated minor	*Manorina flavigula*	Paton and Ford (1977)
*	Bp	Styphelieae	*Brachyloma preissii*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Bp	Styphelieae	*Brachyloma preissii*	Bird	Western spinebill	*Acanthorhynchus superciliosus*	Keighery (1996)
*	Cm	Styphelieae	*Coleanthera myrtoides*	Insect	Bee	*Amegilla pulchra*	Keighery (1996)
*	Cd	Styphelieae	*Conostephium drummondii*	Insect	Bee	*Leioproctus Nodocolletes*	Houston (2000)
*	Cd	Styphelieae	*Conostephium drummondii*	Insect	Bee	*Leioproctus* sp.	Houston and Ladd (2002)
*	Cmi	Styphelieae	*Conostephium minus*	Insect	Bee	*Leioproctus* sp.	Houston and Ladd (2002)
*	Cp	Styphelieae	*Conostephium pendulum*	Insect	Bee	*Amegilla* sp.	Keighery (1996)
*	Cp	Styphelieae	*Conostephium pendulum*	Insect	Bee	*Leioproctus* sp.	Keighery (1996)
*	Cp	Styphelieae	*Conostephium pendulum*	Insect	Bee	*Leioproctus* sp.	Houston and Ladd (2002)
*	Cr	Styphelieae	*Conostephium roei*	Insect	Bee	*Lasioglossum* (*Parasephecodes*) sp.	Houston and Ladd (2002)
*	Cr	Styphelieae	*Conostephium roei*	Insect	Bee	*Leioproctus* sp.	Houston and Ladd (2002)

*	Md	Styphelieae	*Cyathodes dealbata*	Insect	beetle	*Sessinia* sp.	P. McQuillan (University of Tasmania) pers. comm. 2007
*	Cg	Styphelieae	*Cyathodes glauca*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Cg	Styphelieae	*Cyathodes glauca*	Insect	Beetle	Unknown	Hingston and McQuillan (2000)
*	Cg	Styphelieae	*Cyathodes glauca*	Insect	Butterfly	Unknown	Hingston and McQuillan (2000)
*	Cg	Styphelieae	*Cyathodes glauca*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	Cs	Styphelieae	*Cyathodes straminea*	Insect	beetle	Aleocharinae	P. McQuillan (University of Tasmania) pers. comm. 2007
*	Ld	Styphelieae	*Leptecophylla divaricata*	Bird	Black-Headed Honeyeater	*Melithreptus affinis*	Higham and McQuillan (2000)
*	Ld	Styphelieae	*Leptecophylla divaricata*	Bird	Crescent Honeyeater	*Phylidonyris pyrrhoptera*	Higham and McQuillan (2000)
*	Ld	Styphelieae	*Leptecophylla divaricata*	Bird	Eastern Spinebill	*Acanthorhynchus tenuirostris*	Higham and McQuillan (2000)
*	Ld	Styphelieae	*Leptecophylla divaricata*	Insect	Honeybee	*Apis* sp.	Higham and McQuillan (2000)
*	Ld	Styphelieae	*Leptecophylla divaricata*	Bird		Unknown	Hingston and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Bee	*Exoneura* sp.	Higham and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Bee	*Lasioglossum (Austrevylaeus) pertribuarium*	Hingston (1999)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Beetle	*Sessinia* sp.	Higham and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Beetle	Unknown	Hingston and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Blowfly	*Calliphora* sp.	Higham and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Fly	Tachinidae	Higham and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Honeybee	*Apis* sp.	Higham and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Hover Fly	Syrphidae	Higham and McQuillan (2000)
*	Ljp	Styphelieae	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Moth	Unknown	P. McQuillan (University of Tasmania) pers. comm. 2007

*	Ljp	Stypheliea e	*Leptecophylla juniperina* subsp. *parvifolia*	Insect	Wasp	Unknown	Hingston and McQuillan (2000)
*	**Ljj**	**Stypheliea e**	***Leptecophylla juniperina* subsp. *juniperina***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ (Sep 2008)**
*	**Ljj**	**Stypheliea e**	***Leptecophylla juniperina* subsp. *juniperina***	**Insect**	**Bee**	**Unknown**	**KAJ (Sep 2006)**
*	**Ljj**	**Stypheliea e**	***Leptecophylla juniperina* subsp. *juniperina***	**Insect**	**Fly**	**Unknown**	**KAJ (Sep 2006)**
*	**Ljj**	**Stypheliea e**	***Leptecophylla juniperina* subsp. *juniperina***	**Insect**	**Honeybee**	***Apis mellifera***	**KAJ (Sep 2008)**
*	Lf	Stypheliea e	*Leucopogn fraseri*NZ	Insect	Moth	Unknown	Thomson (1926)
*	La	Stypheliea e	*Leucopogon australis*	Insect	Bee	Unknown	Keighery (1996)
*	La	Stypheliea e	*Leucopogon australis*	Insect	Butterfly	Unknown	Keighery (1996)
*	La	Stypheliea e	*Leucopogon australis*	Insect	Fly	Unknown	Keighery (1996)
*	La	Stypheliea e	*Leucopogon australis*	Insect	Moth	Unknown	Keighery (1996)
*	Lc	Stypheliea e	*Leucopogon capitellatus*	Insect	Bee	Unknown	Keighery (1996)
*	Lc	Stypheliea e	*Leucopogon capitellatus*	Insect	Moth	Unknown	Keighery (1996)
*	**Lco**	**Stypheliea e**	***Leucopogon collinus***	**Insect**	**Bee**	***Apis mellifera***	**KAJ (Sep 2009)**
	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Apis mellifera*	P. McQuillan (University of Tasmania) pers. comm. 2008
*	**Lco**	**Stypheliea e**	***Leucopogon collinus***	**Insect**	**Bee**	***Bombus terrestris***	**KAJ (Sep 2009)**
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Callomelitta insularis*	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Euryglossa* (*Euhesma*) sp.	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Exoneura* sp.	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Homalictus niveifrons/megastigmus*	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *brazieri*	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *erythrurum*	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Lasioglossum (Chilalictus) littleri*	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Leioproctus* (*Leioproctus*) sp.	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	*Nomia* (*Austronomia*) sp.	Hingston (1999)
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	**Lco**	**Stypheliea e**	***Leucopogon collinus***	**Insect**	**Butterfly**	**Lycaenidae**	**KAJ (Sep 2008)**
*	Lco	Stypheliea e	*Leucopogon collinus*	Insect	Butterfly	Unknown	Hingston and McQuillan (2000)

	Lco	Styphelieae	*Leucopogon collinus*	Insect	Fly	Calliphoridae	P. McQuillan (University of Tasmania) pers. comm. 2008
*	**Lco**	**Styphelieae**	***Leucopogon collinus***	**Insect**	**Fly**	***Comptosia ocellata***	**KAJ (Sep 2009)**
*	Lco	Styphelieae	*Leucopogon collinus*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	Lco	Styphelieae	*Leucopogon collinus*	Insect	Wasp	Unknown	Hingston and McQuillan (2000)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Euryglossa* (*Euhesma*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Exoneura* sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Exoneura* sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Homalictus niveifrons/megastigmus*	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Austrevylaeus*) *pertribuarium*	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *littleri*	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *opacicolle*	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *orbatum*	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *seductum*	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Ctenonomia*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Leioproctus* (*Leioproctus*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	*Leioproctus* (*Leioproctus*) sp.	Hingston (1999)
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	**Le**	**Styphelieae**	***Leucopogon ericoides***	**Insect**	**Beetle**	***Chauliognathus tricolor***	**KAJ (Sep 2007)**
*	Le	Styphelieae	*Leucopogon ericoides*	Insect	Beetle	Unknown	Hingston and McQuillan (2000)
*	**Le**	**Styphelieae**	***Leucopogon ericoides***	**Insect**	**Beetle**	**Unknown**	**KAJ (Sep 2007)**
*	**Le**	**Styphelieae**	***Leucopogon ericoides***	**Insect**	**Fly**	**Syrphidae**	**KAJ (Sep 2007)**
*	**Le**	**Styphelieae**	***Leucopogon ericoides***	**Insect**	**Fly**	**Tachinidae**	**KAJ (Sep 2007)**
*	Ll	Styphelieae	*Leucopogon lanceolatus*	Insect	Beetle	*Metriorrhynchus rhipidius*	Hawkeswood (2002)
*	Lm	Styphelieae	*Leucopogon montanus*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Lm	Styphelieae	*Leucopogon montanus*	Insect	Beetle	Unknown	Hingston and McQuillan (2000)
*	Lm	Styphelieae	*Leucopogon montanus*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
	Lmu	Styphelieae	*Leucopogon muticus*	Insect	Beetle	*Metriorrhynchus rhipidius*	Hawkeswood (2004)
*	Ln	Styphelieae	*Leucopogon nutans*	Insect	Bee	*Callomelitta antipodes*	Houston (2000)
	Lp	**Styphelieae**	***Leucopogon parviflorus***	**Insect**	**Bee**	***Apis mellifera***	**KAJ (Oct 2007)**
*	**Lp**	**Styphelieae**	***Leucopogon parviflorus***	**Insect**	**Bee**	***Exoneura* sp.**	**KAJ (Oct 2007)**
*	Lp	Styphelieae	*Leucopogon parviflorus*	Insect	Bee	*Homalictus niveifrons/megastigmus*	Hingston (1999)
*	Lp	Styphelieae	*Leucopogon parviflorus*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *brazieri*	Hingston (1999)

*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *littleri*	Hingston (1999)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Bee	*Lasioglossum* (*Parasphecodes*) sp.	Hingston (1999)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Bee	*Nomia* (*Austronomia*) sp.	Hingston (1999)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Bees	Unknown	Keighery (1996)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Butterfly	Unknown	Keighery (1996)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Flies	Unknown	Keighery (1996)
*****	**Lp**	**Stypheliea**	***Leucopogon parviflorus***	**Insect**	**Fly**	***Melangyna* sp.**	**KAJ (Oct 2007)**
*****	**Lp**	**Stypheliea**	***Leucopogon parviflorus***	**Insect**	**Fly**	**Tachinidae**	**KAJ (Oct 2007)**
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Moth	Unknown	Keighery (1996)
*	Lp	Stypheliea	*Leucopogon parviflorus*	Insect	Wasp	*Scolia bimaculata*	Hawkeswood (1993)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Chalicodoma Hackeriapis axillaris*	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Exoneura*	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Lasioglossum Chilalictus castor*	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Leioproctus Leioproctus* sp.	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Leioproctus Leioproctus* sp.	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Leioproctus Leioproctus* sp.	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Leioproctus Leioproctus* sp.	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Leioproctus Leioproctus* sp.	Houston (2000)
*	Lsp	Stypheliea	*Leucopogon sp.*	Insect	Bee	*Leioproctus* sp.	Houston (2000)
*	Lv	Stypheliea	*Leucopogon verticillatus*	Insect	Fly	Unknown	Keighery (1996)
*	Lv	Stypheliea	*Leucopogon verticillatus*	Insect	Mosquito	Unknown	Keighery (1996)
*	Lv	Stypheliea	*Leucopogon verticillatus*	Insect	Tipulid	Unknown	Keighery (1996)
*****	**Lvi**	**Stypheliea**	***Leucopogon virgatus***	**Insect**	**Bee**	***Apis mellifera***	**KAJ (Sep 2008)**
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Bee	*Euryglossa* (*Euhesma*) sp.	Hingston (1999)
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Bee	*Exoneura* sp.	Hingston (1999)
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Bee	*Homalictus niveifrons/megastigmus*	Hingston (1999)
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Bee	*Lasioglossum* (*Chilalictus*) *seductum*	Hingston (1999)
*****	**Lvi**	**Stypheliea**	***Leucopogon virgatus***	**Insect**	**Bee**	**Tachinidae**	**KAJ (Sep 2008)**
*****	**Lvi**	**Stypheliea**	***Leucopogon virgatus***	**Insect**	**Bee**	**Unknown**	**KAJ (Oct 2008)**
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Beetle	*Metriorrhynchus rhipidius*	Hawkeswood (2002)
*	Lvi	Stypheliea	*Leucopogon virgatus*	Insect	Beetle	Unknown	Hingston and McQuillan (2000)
*****	**Lvi**	**Stypheliea**	***Leucopogon virgatus***	**Insect**	**Fly**	**Calliphoridae**	**KAJ (Sep 2008)**
*****	**Lvi**	**Stypheliea**	***Leucopogon virgatus***	**Insect**	**Fly**	**Stratiomyidae**	**KAJ (Sep 2008)**
*****	**Lvi**	**Stypheliea**	***Leucopogon virgatus***	**Insect**	**Fly**	**Syrphidae**	**KAJ (Oct 2008)**

*	**Lvi**	**Styphelieae**	***Leucopogon virgatus***	**Insect**	**Fly**	**Unknown**	**KAJ (Oct 2008)**
*	**Lvi**	**Styphelieae**	***Leucopogon virgatus***	**Insect**	**Butterfly**	**Unknown**	**KAJ (2009)**
*	**Ls**	**Styphelieae**	***Lissanthe strigosa***	**Insect**	**Fly**	**Syriphidae**	**KAJ (Sep 2009)**
*	**Ls**	**Styphelieae**	***Lissanthe strigosa***	**Insect**	**Fly**	**Tachinidae**	**KAJ (Sep 2009)**
*	Me	Styphelieae	*Monotoca elliptica*	Insect	Butterfly	*Neducia mathewi*	P. McQuillan (University of Tasmania) pers. comm. 2007
*	**Mem**	**Styphelieae**	***Monotoca empetrifolia***	**Insect**	**Fly**	**Unknown**	**KAJ (2009)**
*	Mg	Styphelieae	*Monotoca glauca*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Mg	Styphelieae	*Monotoca glauca*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	Ms	Styphelieae	*Monotoca submutica*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Mt	Styphelieae	*Monotoca tamariscina*	Insect	Fly	Unknown	Keighery (1996)
*	Mt	Styphelieae	*Monotoca tamariscina*	Insect	Mosquito	Unknown	Keighery (1996)
*	Pi	Styphelieae	*Pentachondra involucrata*	Insect	Bee	Unknown	Hingston and McQuillan (2000)
*	Pi	Styphelieae	*Pentachondra involucrata*	Insect	Beetle	Unknown	Hingston and McQuillan (2000)
*	Pi	Styphelieae	*Pentachondra involucrata*	Insect	Fly	Unknown	Hingston and McQuillan (2000)
*	Pp	Styphelieae	*Pentachondra pumila*	Insect	Bee	Unknown	P. McQuillan (University of Tasmania) pers. comm. 2007
*	Pp	Styphelieae	*Pentachondra pumila*	Insect	Butterfly	Nymphalidae	P. McQuillan (University of Tasmania) pers. comm. 2007
*	Pp	Styphelieae	*Pentachondra pumila*	Insect	Moth	Geometridae	P. McQuillan (University of Tasmania) pers. comm. 2007
*	Pp	Styphelieae	*Pentachondra pumila*	Insect	Moth	Unknown	Thomson (1926)
	Sa	Styphelieae	*Styphelia albicans*	Insect	honeybee	*Apis mellifera*	Kato and Kawakita (2004)
	Sc	Styphelieae	*Styphelia cymbulae*	Insect	honeybee	*Apis mellifera*	Kato and Kawakita (2004)
	Sf	Styphelieae	*Styphelia floribunda*	Insect	honeybee	*Apis mellifera*	Kato and Kawakita (2004)
*	Sh	Styphelieae	*Styphelia hainesii*	Bird	Brown headed honeyeater	*Melithreptus brevirostris*	Keighery (1996)
*	Sh	Styphelieae	*Styphelia hainesii*	Bird	Tawny crowned honeyeater	*Phylidonyris melanops*	Keighery (1996)
*	St	Styphelieae	*Styphelia tenuifolia*	Insect	Butterfly	Unknown	Keighery (1996)
*	St	Styphelieae	*Styphelia tenuifolia*	Insect	Moth	Unknown	Keighery (1996)
*	**Tc**	**Styphelieae**	***Trochocarpa cunninghamii***	**Bird**	**Eastern spinebill**	***Acanthorhynchus tenuirostris***	**Johnson *et al.* (2010); Chapter 2**
	Tg	**Styphelieae**	***Trochocarpa gunnii***	**Insect**	**Bee**	***Apis mellifera***	**KAJ (Mar 2007)**
*	**Tg**	**Styphelieae**	***Trochocarpa gunnii***	**Insect**	**Butterfly**	**Graphium macleayanus**	**KAJ (Mar 2007)**
*	**Tg**	**Styphelieae**	***Trochocarpa gunnii***	**Insect**	**Fly**	**Syrphidae**	**KAJ (Feb 2008)**
*	**Tg**	**Styphelieae**	***Trochocarpa gunnii***	**Insect**	**Fly**	**Tachinidae**	**KAJ (Feb 2009)**

*	Tt	Styphelieae	*Trochocarpa thymifolia*	Bird	Crescent honeyeater	*Phylidonyris pyrrhoptera*	KAJ (Mar 2007)
*	Tt	Styphelieae	*Trochocarpa thymifolia*	Bird	Strong billed honeyeater	*Melithreptus validirostris*	KAJ (Mar 2007)

Appendix 7-3 Floral trait and potential pollinators of epacrids - data sets

Table A. Flora trait profiles for epacrid species (1 = trait present, 0 = trait absent, , B = blue corolla, Pu = purple corolla, R = red corolla, Pi = pink corolla, Wh = white corolla, G = green corolla, Y = yellow corolla, Br = brush flower, Lt = long tube (10+ mm), Mt = medium tube (5 to <10 mm), St = short tube (<5 mm), BeCu = bell/cup shape flower, C = constricted corolla, Na = narrow corolla, w = wide corolla, N = nectar)

Code	Species	Floral trait															
		B	Pu	R	Pi	Wh	G	Y	Br	Lt	Mt	St	BeCu	Vc	Na	W	N
Ac	*Acrotriche cordata*	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	1
Aci	*Astroloma ciliatum*	0	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1
Aco	*Astroloma conostephioides*	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1
Acom	*Astroloma compactum*	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1
Adr	*Astroloma drummondii*	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1
Ae	*Astroloma epacridis*	0	0	1	1	1	0	0	0	1	0	0	0	0	1	0	1
Afo	*Astroloma foliosum*	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	1
Ag	*Andersonia grandiflora*	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	1
Agl	*Astroloma glaucescens*	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1
Ah	*Andersonia heterophylla*	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1
Am	*Andersonia micrantha*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Ama	*Astroloma macrocalyx*	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
Ami	*Astroloma microcalyx*	0	0	1	0	1	0	0	0	1	0	0	0	1	0	0	1
Apa	*Astroloma pallidum*	0	0	1	1	1	0	0	0	1	1	0	0	1	0	0	1
Apr	*Astroloma prostratum*	0	0	1	1	0	1	1	0	1	0	0	0	1	0	0	1
Ase	*Andersonia setifolia*	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	1
Aser	*Astroloma serratifolium*	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0	1
Asp	*Andersonia sprengeloides*	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
Ax	*Astroloma xerophyllum*	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
Bd	*Brachyloma daphnoides*	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	1
Be	*Brachyloma ericoides*	0	0	0	1	1	0	0	0	0	0	1	0	1	1	0	1
Bp	*Brachyloma preissii*	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1
Cd	*Conostephium drummondii*	0	1	1	1	1	0	0	0	1	0	0	0	1	0	0	1
Cg	*Cyathodes glauca*	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1
Cm	*Coleanthera myrtoides*	0	1	0	1	1	0	0	0	0	0	0	1	0	0	1	0
Cmi	*Conostephium minus*	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1
Cp	*Conostephium pendulum*	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	1
Cr	*Conostephium roei*	0	1	1	0	1	0	0	0	1	0	0	0	1	0	0	1
Cru	*Cosmelia rubra*	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1
Cs	*Cyathodes straminea*	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0	1
Da	*Dracophyllum acerosum*	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1
Dac	*Dracophyllum acicularifolium*	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
Dm	*Dracophyllum minimum*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Dp	*Dracophyllum pronum*	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1
Dr	*Dracophyllum rosmarinifolium*	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
Dra	*Dracophyllum ramosum*	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0	1
Du	*Dracophyllum*	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1

	uniflorum																
Ec	*Epacris corymbifolia*	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1
Ei	*Epacris impressa*	0	0	1	1	1	0	0	0	1	1	0	0	0	0	1	1
El	*Epacris lanuginosa*	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
Em	*Epacris marginata*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Ep	*Epacris paludosa*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Epe	*Epacris petrophila*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Es	*Epacris serpyllifolia*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Est	*Epacris stuartii*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
La	*Leucopogon australis*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Lc	*Leucopogon capitellatus*	0	0	0	0	1	0	0	0	0	0	1	1	1	0	0	1
Lci	*Lysinema ciliatum*	0	0	0	1	1	0	0	0	1	0	0	0	1	0	0	1
Lco	*Leucopogon collinus*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Ld	*Leptecophylla divaricata*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Le	*Leucopogon ericoides*	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	1
Lel	*Lysinema elegans*	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	1
Lf	*Lysinema fimbriatum*	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
Ljj	*Leptecophylla juniperina* var. *juniperina*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Ljp	*Leptecophylla juniperina subsp. parvifolia*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Ll	*Leucopogon lanceolatus*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Lm	*Leucopogon montanus*	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1
Ln	*Leucopogon nutans*	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
Lp	*Leucopogon parviflorus*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Ls	*Lissanthe strigosa*	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	1
Lv	*Leucopogon verticillatus*	0	0	1	1	0	0	0	0	0	1	1	0	0	1	0	1
Lvi	*Leucopogon virgatus*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Md	*Montitega dealbata*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Me	*Monotoca elliptica*	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Mem	*Monotoca empetrifolia*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Mg	*Monotoca glauca*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Ms	*Monotoca submutica*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Mt	*Dielsiodoxa tamariscina*	0	0	0	0	1	1	0	0	0	0	1	1	0	0	1	0
Np	*Needhamiella pumilio*	0	0	1	1	1	0	0	0	0	0	1	1	1	0	0	1
Pc	*Prionotes cerinthoides*	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1
Pi	*Pentachondra involucrata*	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	1
Pp	*Pentachondra pumila*	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	1
Ra	*Richea acerosa*	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1
Rc	*Richea contentinalis*	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
Rd	*Richea dracophylla*	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
Rm	*Richea milliganii*	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1
Rp	*Richea pandanifolia*	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1
Rpr	*Richea procera*	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Rs	*Richea scoparia*	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	1
Rsp	*Richea sprengelioides*	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Sh	*Styphelia hainesii*	0	0	1	0	0	1	0	0	1	0	0	0	0	1	0	1
Si	*Spengelia incarnata*	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0
Sp	*Spengelia propinqua*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0
St	*Styphelia tenuifolia*	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	1
Tc	*Trochocarpa cunninghamii*	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1
Tg	*Trochocarpa gunnii*	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Tt	*Trochocarpa thymifolia*	0	0	1	1	0	0	0	0	0	0	0	1	0	0	1	1

Table B. Potential pollinator profiles for epacrid species

Code	Species	Potential pollinator						
		Fly	Bee	Beetle	Butterfly	Wasp	Bird	Moth
Ac	*Acrotriche cordata*	1	0	0	0	0	0	0
Aci	*Astroloma ciliatum*	0	0	0	0	0	1	0
Aco	*Astroloma conostephioides*	0	0	0	0	0	1	0
Acom	*Astroloma compactum*	0	0	0	0	0	1	0
Adr	*Astroloma drummondii*	0	0	0	0	0	1	0
Ae	*Astroloma epacridis*	0	0	0	0	0	1	0
Afo	*Astroloma foliosum*	0	0	0	0	0	1	0
Ag	*Andersonia grandiflora*	0	0	0	0	0	1	0
Agl	*Astroloma glaucescens*	0	0	0	0	0	1	0
Ah	*Andersonia heterophylla*	0	1	0	0	0	0	0
Am	*Andersonia micrantha*	1	0	0	0	0	0	0
Ama	*Astroloma macrocalyx*	0	0	0	0	0	1	0
Ami	*Astroloma microcalyx*	0	0	0	0	0	1	0
Apa	*Astroloma pallidum*	0	0	0	0	0	1	0
Apr	*Astroloma prostratum*	0	0	0	0	0	1	0
Ase	*Andersonia setifolia*	0	0	0	0	0	1	0
Aser	*Astroloma serratifolium*	0	0	0	0	0	1	0
Asp	*Andersonia sprengeloides*	0	0	0	1	0	0	0
Ax	*Astroloma xerophyllum*	0	1	0	0	0	0	0
Bd	*Brachyloma daphnoides*	0	0	1	0	0	0	0
Be	*Brachyloma ericoides*	0	0	0	0	0	1	0
Bp	*Brachyloma preissii*	0	0	0	0	0	1	0
Cd	*Conostephium drummondii*	0	1	0	0	0	0	0
Cg	*Cyathodes glauca*	1	1	1	1	0	0	0
Cm	*Coleanthera myrtoides*	0	1	0	0	0	0	0
Cmi	*Conostephium minus*	0	1	0	0	0	0	0
Cp	*Conostephium pendulum*	0	1	0	0	0	0	0
Cr	*Conostephium roei*	0	1	0	0	0	0	0
Cru	*Cosmelia rubra*	0	0	0	0	0	1	0
Cs	*Cyathodes straminea*	0	0	1	0	0	0	0
Da	*Dracophyllum acerosum*	0	0	0	1	0	0	1
Dac	*Dracophyllum acicularifolium*	0	0	1	0	0	0	0
Dm	*Dracophyllum minimum*	1	0	1	1	0	0	0
Dp	*Dracophyllum pronum*	1	0	0	1	0	0	1
Dr	*Dracophyllum rosmarinifolium*	0	0	0	0	0	0	1
Dra	*Dracophyllum ramosum*	0	0	1	1	0	0	0
Du	*Dracophyllum uniflorum*	0	0	0	0	0	0	1
Ec	*Epacris corymbifolia*	0	0	0	1	0	0	0
Ei	*Epacris impressa*	1	1	1	1	0	1	0
El	*Epacris lanuginosa*	1	1	0	1	0	0	0
Em	*Epacris marginata*	1	1	0	0	0	0	0
Ep	*Epacris paludosa*	0	0	0	1	0	0	0
Epe	*Epacris petrophila*	0	0	0	1	0	0	0
Es	*Epacris serpyllifolia*	1	0	1	1	0	0	0
Est	*Epacris stuartii*	1	0	0	0	0	0	0
La	*Leucopogon australis*	1	1	0	1	0	0	1
Lc	*Leucopogon capitellatus*	0	1	0	0	0	0	1
Lci	*Lysinema ciliatum*	0	0	0	0	0	0	1
Lco	*Leucopogon collinus*	1	1	0	1	1	0	0
Ld	*Leptecophylla divaricata*	0	0	0	0	0	1	0
Le	*Leucopogon ericoides*	1	1	1	0	0	0	0
Lel	*Lysinema elegans*	0	0	0	0	0	0	1

Lf	*Lysinema fimbriatum*	0	0	0	1	0	0	0
Ljj	*Leptecophylla juniperina* var. *juniperina*	1	1	0	0	0	0	0
Ljp	*Leptecophylla juniperina subsp. parvifolia*	1	1	1	1	1	0	1
Ll	*Leucopogon lanceolatus*	0	0	1	0	0	0	0
Lm	*Leucopogon montanus*	1	1	1	0	0	0	0
Ln	*Leucopogon nutans*	0	1	0	0	0	0	0
Lp	*Leucopogon parviflorus*	1	1	0	1	1	0	1
Ls	*Lissanthe strigosa*	1	0	0	0	0	0	0
Lv	*Leucopogon verticillatus*	1	0	0	0	0	0	0
Lvi	*Leucopogon virgatus*	1	1	1	1	0	0	0
Md	*Montitega dealbata*	0	0	1	0	0	0	0
Me	*Monotoca elliptica*	0	0	0	1	0	0	0
Mem	*Monotoca empetrifolia*	1	0	0	0	0	0	0
Mg	*Monotoca glauca*	1	1	0	0	0	0	0
Ms	*Monotoca submutica*	0	1	0	0	0	0	0
Mt	*Dielsiodoxa tamariscina*	1	0	0	0	0	0	0
Np	*Needhamiella pumilio*	0	0	0	1	0	0	1
Pc	*Prionotes cerinthoides*	0	0	0	0	0	1	0
Pi	*Pentachondra involucrata*	1	1	1	0	0	0	0
Pp	*Pentachondra pumila*	0	1	0	1	0	0	1
Ra	*Richea acerosa*	1	1	0	0	0	0	0
Rc	*Richea contentinalis*	1	1	0	0	0	0	0
Rd	*Richea dracophylla*	1	1	0	0	1	1	0
Rm	*Richea milliganii*	1	1	0	0	1	0	0
Rp	*Richea pandanifolia*	1	1	1	0	0	1	0
Rpr	*Richea procera*	1	1	0	0	0	0	0
Rs	*Richea scoparia*	1	0	1	0	1	0	0
Rsp	*Richea sprengelioides*	1	1	1	0	0	0	0
Sh	*Styphelia hainesii*	0	0	0	0	0	1	0
Si	*Spengelia incarnata*	0	1	0	0	0	0	0
Sp	*Spengelia propinqua*	1	1	0	0	0	0	0
St	*Styphelia tenuifolia*	0	0	0	1	0	0	1
Tc	*Trochocarpa cunninghamii*	0	0	0	0	0	1	0
Tg	*Trochocarpa gunnii*	1	1	0	1	0	0	0
Tt	*Trochocarpa thymifolia*	0	0	0	0	0	1	0

Table C. Flora trait profiles for epacrid genera (1 = trait present, 0 = trait absent, B = blue corolla, Pu = purple corolla, R = red corolla, Pi = pink corolla, Wh = white corolla, G = green corolla, Y = yellow corolla, Br = brush flower, Lt = long tube (10+ mm), Mt = medium tube (5 to <10 mm), St = short tube (<5 mm), BeCu = bell/cup shape flower, C = constricted corolla, Na = narrow corolla, w = wide corolla, N = nectar)

Genus	Floral trait															
	B	Pu	R	Pi	Wh	G	Y	Br	Lt	Mt	St	BeCu	C	Na	W	N
Acrothamnus	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1
AcrotricheA	0	0	0	0	0	1	0	0	0	0	1	0	1	1	0	1
AcrotricheB	0	0	0	0	1	0	0	0	0	0	1	0	1	1	0	1
AcrotricheD	0	1	0	0	0	0	0	0	0	0	1	0	1	1	0	1
AcrotricheC	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	1
Agioritia	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1
AndersoniaA	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1
AndersoniaB	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1
AndersoniaC	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0	1
AndersoniaD	0	0	0	0	1	0	1	0	0	1	1	0	0	1	0	1
Androstoma	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
ArcheriaA	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1
ArcheriaB	0	0	0	0	1	0	0	0	0	1	0	0	0	1	0	1
ArcheriaC	0	0	1	0	0	0	0	0	0	1	0	0	0	1	0	1
AstrolomaA	0	0	1	1	0	0	0	0	1	1	0	0	1	0	0	1
AstrolomaB	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
AstrolomaC	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	1
AstrolomaD	0	0	0	0	0	1	1	0	1	1	0	0	1	0	0	1
AstrolomaE	0	1	0	0	0	0	0	0	1	0	0	0	1	0	0	1
BrachylomaA	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	1
BrachylomaB	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0	1
Budawangia	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0
ColeantheraA	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0
ColeantheraB	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	0
ColeantheraC	0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0
ConostephiumA	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0
ConostephiumB	0	0	1	1	1	0	0	0	1	0	0	0	1	0	0	0
ConostephiumC	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0
Cosmelia	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1
Croninia	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
Cyathodes	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1	1
Cyathopsis	0	0	1	1	1	0	0	0	0	1	1	0	0	0	1	1
Decatoca	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Dielsiodoxa	0	0	0	0	1	1	1	0	0	0	0	1	0	0	1	1
DracophyllumA	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1
DracophyllumB	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1
DracophyllumC	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
DracophyllumD	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1
EpacrisA	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1
EpacrisB	0	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1
EpacrisC	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Lebetanthus	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	1
LeptecophyllaA	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	1

LeptecophyllaB	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1
LeucopogonA	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1
LeucopogonB	0	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1
LeucopogonC	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1
LeucopogonD	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1
LeucopogonE	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	1
LeucopogonF	0	0	0	0	0	1	1	0	0	1	0	0	0	1	1	1
Lissanthe	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1
Lysinema	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
MelichrusA	0	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1
MelichrusB	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1
MelichrusC	0	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1
Monotoca	0	0	0	0	1	0	0	0	0	0	0	1	0	0	1	1
Montitega	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	1
Needhamiella	0	0	1	0	1	0	0	0	0	0	1	0	0	1	0	1
Oligarrhena	0	0	0	0	1	0	1	0	0	0	1	0	0	0	1	1
PentachondraA	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1
PentachondraB	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1
Planocarpa	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	1
Priontoes	0	0	1	1	0	0	0	0	1	0	0	0	0	0	1	1
Pseudactinia	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1
RicheaA	0	0	1	1	0	0	0	1	0	0	0	0	0	0	0	1
RicheaB	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
RicheaC	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
Rupicola	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	0
Sphenotoma	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	1
Sprengelia	0	0	0	1	1	0	0	0	0	0	1	1	0	0	1	0
StypheliaA	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1
StypheliaB	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	1
StypheliaC	0	0	0	0	0	1	1	0	1	0	0	0	0	0	1	1
TrochocarpaA	0	0	1	1	0	0	0	0	0	0	1	0	0	0	1	1
TrochocarpaB	0	0	0	0	1	0	0	0	0	0	1	1	0	0	1	1
Woollsia	0	0	0	1	1	0	0	0	1	1	0	0	1	0	0	1

Appendix 7-4 Divisions within genera for Random Forests analysis

Group name	Description	Example species	Reference
*Acrotriche*A	Green corolla, short tube.	*A. cordata*, *A. depressa*, *A. patula, A. serrulata*	Jessop and Toelken (1986), Paczkowska and Chapman (2000), Curtis (1963), Walsh and Entwisle (1996), Harden (1992)
*Acrotriche*B	White corolla, short tube.	*A. affinis*, *A. cordata*	
*Acrotiche*C	Purple corolla, short tube.	*A. ramiflora*	
*Acrotriche*D	Pink corolla, medium tube.	*A. fasciculiflora*, *A. halmaturina*	
*Andersonia*A	Blue corolla or purple corolla.	*A. auriculata*, *A. caerulea*, *A. hammersleyana*	FloraBase WA
*Andersonia*B	Red corolla.	*A. setifolia*	
*Andersonia*C	Pink corolla.	*A. brevifolia*, *A. gracilis*, *A. simplex*	
*Andersonia*D	White or yellow corolla.	*A. annelsii*, *A. axilliflora*, *A. bifida*	
*Archeria*A	Pink corolla.	*A. comberi*, *A. racemosa*	Curtis (1963), Allan (1961)
*Archeria*B	White corolla.	*A. serpyllifolia*	
*Archeria*C	Red corolla.	*A. traversii*	
*Astroloma*A	Red or pink corolla, long or medium tube.	*A. humifusum*, *A. conostephioides*, *A. baxteri*	Curtis (1963), Harden (1992), Walsh and Entwisle (1996), FloraBase WA
*Astroloma*B	White corolla, long tube.	*A. macrocalyx*, *A. pallidum*	
*Astroloma*C	White corolla, short or medium tube.	*A. xerophyllum*	
*Astroloma*D	Yellow or green corolla, long or medium tube.	*A. pinifolium*, *A. pallidum*	
*Astroloma*E	Purple flower, long tube	*A. ciliatum*	
*Brachyloma*A	Red or pink corolla, short tube.	*B. ericoides*, *B. mogin*, *B. preissii*	Curtis (1963), Harden (1992), Walsh and Entwisle (1996), FloraBase WA
*Brachyloma*B	White or green corolla, short tube.	*B. daphnoides*, *B. depressum*, *B. Ciliatum*, *B. scortechinii*	
*Coleanthera*A	Pink corolla.	*C. myrtoides*, *C. coelophylla*	FloraBase WA
*Coleanthera*B	White corolla.	*C. myrtoides*, *C. coelophylla*	
*Coleanthera*C	Purple corolla.	*C. myrtoides*	
*Constephium*A	Blue or purple and white corolla.	*C. magnum*, *C. pendulum*, *C. preisii*	FloraBase WA Note that all *Conostephium* spp. are polymorphic for colour.
*Conostephium*B	Red or pink and white corolla.	*C. marchantiorum*, *C. pendulum*, *C. preisii*, *C. magnum*, *C. preisii*	
*Conostephium*C	White or yellow corolla.	*C. marchantiorum*, *C. pendulum*, *C. preisii*	
*Dracophyllum*A	Pink, long or medium tube.	*D. macranthum*, *D. ouaiemense*	Wagstaff et al. (2010), Brown and Streiber (1999); Curtis (1963)
*Dracophyllum*B	White corolla, medium or short tube.	*D. secundum*	
*Dracophyllum*C	White corolla, short tube.	*D. minimum*	
*Dracophyllum*D	Pink corolla, short tube.	*D. milliganii*	

Group name	Description	Example species	Reference
*Epacris*A	Red corolla, long tube.	*E. impressa*, *E. reclinata*, *E. longiflora*	Curtis (1963), Harden (1992), Walsh and Entwisle (1996)
*Epacris*B	Pink or white corolla, long or medium tube.	*E. impressa*, *E. crassifolia*, *E. robusta*, *E. obtusifolia*, *E. longiflora*	
*Epacris*C	White corolla, short tube or cup-shape.	*E. marginata*, *E. serpyllifolia*, *E. gunnii*, *E. acuminata*	
*Leptecophylla*A	White corolla, medium or short tube, wide corolla mouth.	*L. divaricata*, *L. pendulosa*	Curtis (1963)
*Leptecophylla*B	White corolla, short tube, wide or narrow corolla mouth.	*L. juniperina*, *L. pogonocalyx*	
*Leucopogon*A	Pink corolla, medium tube.	*L. fletcheri*, *L. ericoides*	Curtis (1963), Harden (1992), Walsh and Entwisle (1996) Note that *L. rufus* flowers go red upon drying (Walsh and Entwisle, 1996) but are white when fresh. Thus, no red included.
*Leucopogon*B	Pink corolla short tube.	*L. thymifolius*, *L. ericoides*	
*Leucopogon*C	White corolla, medium tube.	*L. juniperinus*, *L. neo-anglicus*, *L. cordifolius*	
*Leucopogon*D	White corolla, short tube.	*L. amplexicaulis*, *L. pilifer*, *L. collinus*, *L. ericoides*	
*Leucopogon*E	Red corolla, long tube.	*L. oxycedrus*	
*Leucopogon*F	Yellow or green corolla, medium tube.	*L. crassifolius*	
*Melichrus*A	Red or pink corolla; short tube or cup-shape.	*M. erubescens*	Harden (1992)
*Melichrus*B	White corolla, short tube or cup-shape.	*M. procumbens*, *M. adpressus*, *M. urceolatus*	
*Melichrus*C	Green or yellow corolla, short tube.	*M. adpressus*, *M. urceolatus*	
*Pentachondra*A	Red or pink corolla, medium or short tube.	*P. pumila*, *P. involucrata*	Curtis (1963), Harden (1992), Walsh and Entwisle (1996), Mark and Adams (1986)
*Pentachondra*B	White corolla, medium tube.	*P. involucrata*, *P. pumila*, *P. ericaefolia*, *P. dehiscens*	
*Richea*A	Red or pink stamens, complex inflorescence.	Species from section *Dracophylloides* – *R. scoparia*, *R. alpina*, *R. pandanifolia*	Curtis (1963), Walsh and Entwisle (1996)
*Richea*B	White stamens, simple or complex inflorescence.	Species from section *Dracophylloides* or *Cystanthe* – *R. acerosa*, *R. dracophylla*	
*Richea*C	Yellow or green stamens, simple inflorescence, nectarless flowers.	Species from section *Cystanthe* – *R. procera*, *R. sprengelioides*	
*Styphelia*A	Red corolla.	*S. tubiflora*, *S. psiloclada*, *S. triflora*, *S. laeta*	Curtis (1963), Harden (1992), Walsh and Entwisle (1996) Most can be less frequently coloured as for StypheliaB. *S. perileuca* is predominantly yellow-green but has fine red stripes.
*Styphelia*B	Cream corolla.	*S. adscendens*, *S. angustifolia*	
*Styphelia*C	Green or yellow corolla.	*S. adscendens*, *S. laeta*, *S. longifolia*	

Group name	Description	Example species	Reference
*Trochocarpa*A	Red or pink flowers, hairy flowers.	*T. thymifolia*, *T. cunninghammi*, *T. clarkei* (flowers are bi-coloured maroon above and green below).	Curtis (1963), Harden (1992), Walsh and Entwisle (1996), Van Royen (1982)
*Trochocarpa*B	White flowers, glabrous or hairy.	*T. gunnii*, *T. bellendenkerensis*, *T. laurina*	

Appendix 7-5 Predicted pollinators – Random Forests

Table A. Species predicted for bird pollination by RF analysis (2 = bird, 1 = not bird)

Ac	Aci	Aco	Acom	Adr	Ae	Afo	Ag	Agl	Ah
1	2	2	2	2	2	2	2	2	1
Am	Ama	Ami	Apa	Apr	Ase	Aser	Asp	Ax	Bd
1	1	2	2	2	2	2	1	1	1
Be	Bp	Cd	Cg	Cm	Cmi	Cp	Cr	Cru	Cs
1	2	1	1	1	1	1	1	2	1
Da	Dac	Dm	Dp	Dr	Dra	Du	Ec	Ei	El
1	1	1	1	1	1	1	1	2	1
Em	Ep	Epe	Es	Est	La	Lc	Lci	Lco	Ld
1	1	1	1	1	1	1	1	1	1
Le	Lel	Lf	Ljj	Ljp	Ll	Lm	Ln	Lp	Ls
1	1	1	1	1	1	1	1	1	1
Lv	Lvi	Md	Me	Mem	Mg	Ms	Mt	Np	Pc
1	1	1	1	1	1	1	1	1	2
Pi	Pp	Ra	Rc	Rd	Rm	Rp	Rpr	Rs	Rsp
1	1	1	1	1	1	2	1	1	1
Sh	Si	Sp	St	Tc	Tg	Tt			
2	1	1	1	1	1	2			

Table B. Species predicted for fly/bee pollination by RF analysis (2 = fly and/or bee, 1 = not fly or bee)

Ac	Aci	Aco	Acom	Adr	Ae	Afo	Ag	Agl	Ah
2	1	1	1	1	1	1	1	1	2
Am	Ama	Ami	Apa	Apr	Ase	Aser	Asp	Ax	Bd
2	1	1	1	1	1	1	1	1	1
Be	Bp	Cd	Cg	Cm	Cmi	Cp	Cr	Cru	Cs
1	1	2	2	2	2	2	2	1	1
Da	Dac	Dm	Dp	Dr	Dra	Du	Ec	Ei	El
1	1	2	2	1	1	1	1	2	1
Em	Ep	Epe	Es	Est	La	Lc	Lci	Lco	Ld
2	2	2	2	2	2	2	1	2	2
Le	Lel	Lf	Ljj	Ljp	Ll	Lm	Ln	Lp	Ls
2	1	1	2	2	2	2	1	2	2
Lv	Lvi	Md	Me	Mem	Mg	Ms	Mt	Np	Pc
2	2	2	2	2	2	2	2	1	1
Pi	Pp	Ra	Rc	Rd	Rm	Rp	Rpr	Rs	Rsp
2	2	2	2	2	2	2	2	2	2
Sh	Si	Sp	St	Tc	Tg	Tt			
1	2	2	1	2	2	1			

Table C. Genus predicted for bird pollination by RF analysis (2 = bird, 1 = not bird)

Acrothamnus	*Acrotriche*A	*Acrotriche*B	*Acrotriche*D	*Acrotriche*C	*Agioritia*
1	1	1	1	1	1
*Andersonia*A	*Andersonia*B	*Andersonia*C	*Andersonia*D	*Androstoma*	*Archeria*A
1	2	1	1	1	1
*Archeria*B	*Archeria*C	*Astroloma*A	*Astroloma*B	*Astroloma*C	*Astroloma*D
1	2	2	1	1	1
*Astroloma*E	*Brachyloma*A	*Brachyloma*B	*Budawangia*	*Coleanthera*A	*Coleanthera*B
1	1	1	1	1	1
*Coleanthera*C	*Conostephium*A	*Conostephium*B	*Conostephium*C	*Cosmelia*	*Croninia*
1	1	2	1	2	1
Cyathodes	*Cyathopsis*	*Decatoca*	*Dielsiodoxa*	*Dracophyllum*A	*Dracophyllum*B
1	2	1	1	1	1
*Dracophyllum*C	*Dracophyllum*D	*Epacris*A	*Epacris*B	*Epacris*C	*Lebetanthus*
1	1	2	1	1	1
*Leptecophylla*A	*Leptecophylla*B	*Leucopogon*A	*Leucopogon*B	*Leucopogon*C	*Leucopogon*D
1	1	1	1	1	1
*Leucopogon*E	*Leucopogon*F	*Lissanthe*	*Lysinema*	*Melichrus*A	*Melichrus*B
2	1	1	1	2	1
*Melichrus*C	*Monotoca*	*Montitega*	*Needhamiella*	*Oligarrhena*	*Pentachondra*A
1	1	1	1	1	1
*Pentachondra*B	*Planocarpa*	*Priontoes*	*Pseudactinia*	*Richea*A	*Richea*B
1	1	2	1	2	1
*Richea*C	*Rupicola*	*Sphenotoma*	*Sprengelia*	*Styphelia*A	*Styphelia*B
1	1	1	1	2	1
*Styphelia*C	*Trochocarpa*A	*Trochocarpa*B	*Woollsia*		
1	2	1	1		

Table D. Genus predicted for fly/bee pollination by RF analysis (2 = fly and/or bee, 1 = not fly or bee)

Acrothamnus	*Acrotriche*A	*Acrotriche*B	*Acrotriche*D	*Acrotriche*C	*Agioritia*
2	2	1	2	1	2
*Andersonia*A	*Andersonia*B	*Andersonia*C	*Andersonia*D	*Androstoma*	*Archeria*A
1	2	2	1	2	2
*Archeria*B	*Archeria*C	*Astroloma*A	*Astroloma*B	*Astroloma*C	*Astroloma*D
2	2	1	1	1	1
*Astroloma*E	*Brachyloma*A	*Brachyloma*B	*Budawangia*	*Coleanthera*A	*Coleanthera*B
1	1	2	2	2	2
*Coleanthera*C	*Conostephium*A	*Conostephium*B	*Conostephium*C	*Cosmelia*	*Croninia*
2	2	1	1	1	1
Cyathodes	*Cyathopsis*	*Decatoca*	*Dielsiodoxa*	*Dracophyllum*A	*Dracophyllum*B
1	1	2	2	1	1
*Dracophyllum*C	*Dracophyllum*D	*Epacris*A	*Epacris*B	*Epacris*C	*Lebetanthus*
2	1	1	1	2	2
*Leptecophylla*A	*Leptecophylla*B	*Leucopogon*A	*Leucopogon*B	*Leucopogon*C	*Leucopogon*D
1	2	1	1	1	2
*Leucopogon*E	*Leucopogon*F	*Lissanthe*	*Lysinema*	*Melichrus*A	*Melichrus*B
1	2	2	1	1	2
*Melichrus*C	*Monotoca*	*Montitega*	*Needhamiella*	*Oligarrhena*	*Pentachondra*A
2	2	2	2	2	1
*Pentachondra*B	*Planocarpa*	*Priontoes*	*Pseudactinia*	*Richea*A	*Richea*B
1	2	1	2	2	2
*Richea*C	*Rupicola*	*Sphenotoma*	*Sprengelia*	*Styphelia*A	*Styphelia*B
2	2	1	2	1	1
*Styphelia*C	*Trochocarpa*A	*Trochocarpa*B	*Woollsia*		
1	1	2	1		

Chapter 8 Conclusion and future directions

Epacrid floral traits were related to bird, bee, and fly pollination systems within a modern phylogenetic framework. Birds were found to have a strong association with the red- and long-tubed epacrids. This association held up to scrutiny on a number of levels, namely the breeding system and pollinators of *Prionotes*, which revealed this as a basal association in the epacrids (Johnson et al., 2010 (Chapter 2)) and in the statistical classification of pollination syndromes for the entire subfamily (Chapter 7). This finding is consistent with the results of previous research from across the globe on the association between red flowers and bird pollination (Grant, 1966; Paige and Whitham, 1985; Armesto et al., 1996; Schemske and Bradshaw, 1999; Rodríguez-Gironés, 2004; Cronk and Ojeda, 2008). However, as with most pollination syndromes there are frequent exceptions or qualifications. For epacrids these were bird visitation to *Richea* brush flowers and certain smaller and lighter-coloured tubular flowers, including *Brachyloma* and *Leptecophylla.*

In contrast to bird pollination, bee- and fly-pollinated epacrids tend not to have red flowers and long floral tubes in their pollination syndromes. The pollination ecology of *Richea* species revealed that brush flowers were strongly related to both bees and flies (Chapter 5). Similar to other brush flowers from important Australian plant families such as the Proteaceae and Myrtaceae (Hingston and McQuillan, 2000), the flowers of *Richea* were found to have generalised pollination systems (Chapter 5). The *Sprengelia* study (Chapter 4) confirmed the suspicions of Houston and Ladd (2002) that sonication does occur in *Sprengelia.* Houston and Ladd (2002) confirmed that pollen was collected via sonication from *Conostephium* in the tribe Styphelieae. Now, it has been confirmed that pollen is also collected by sonication from *Sprengelia incarnata* in the tribe Cosmelieae (Chapter 4). However, *S. propinqua* was not found to be sonicated but rather had its pollen collected by scraping.

The introduced honeybee *Apis mellifera* foraged at *S. propinqua* but ignored *S. incarnata*. Selective foraging by the honeybee has potential implications on the reproduction and evolution of the *Sprengelia* species. Honeybees and bumblebees occurred across all environments surveyed and foraged on species from *Brachyloma*, *Dracophyllum*, *Epacris*, *Leptecophylla*, *Leucopogon*, *Richea*, *Prionotes*, *Sprengelia Styphelia*, and *Trochocarpa*. Their

interactions with epacrids were variable, ranging from nectar-robbing in the long-tubed *Prionotes* and *E. impressa* to acting as potential pollinators in some shorter corolla species including *E. marginata.* While there can be no doubt that introduced bees are impacting on native fauna and plant ecology (Hingston and McQuillan, 1998; Hingston, 2006; Hingston et al., 2006; Hingston, 2007), it is probable that in the future they will be a driver of evolution of the Australian flora.

In addition to introduced bees, introduced rodents are also impacting on epacrids. The native pollinator(s) of *Acrotriche serrulata* is(are) still to be discovered (Schneemilch and Steggles, 2010; Johnson et al., 2011 (Chapter 3); Schneemilch et al., 2011). However, there is evidence that in some locations the introduced black rat (*Rattus rattus*) has moved into the niche. If this is confirmed, then there are implications for the long-term reproduction and conservation of *A. serrulata* and potentially other native plants. Fenster and Martén-Rodríguez (2007) postulate that pollinator limitation will favour evolutionary shifts in pollination systems from specialized to generalized or vice versa and may result in selection of floral traits to attract alternative or additional pollinators. Although advances have been made in our knowledge of the interactions between mammals and epacrids their effectiveness as pollinators remains uncertain. For example, *Andersonia*, *Astroloma* and *Leucopogon* provide minor sources of nectar for the honey possum (Keighery, 1996), while *Acrotriche serrulata* provides a source of food for brown anthechinus (*Antechinus stuartii*) (Fletcher, 1977), common brushtail possum (*Trichosurus vulpecula*), and black rat (Johnson et al. 2011 (Chapter 3)). Having established mammals as potential pollinators, the next step is to show that they can transport pollen between conspecific epacrids and lead to their successful reproduction. Much of the Australian fauna are nocturnal, including most native mammals; however, very little work has been done on nocturnal pollination systems.

Although skinks (*Niveoscinsus* spp.) are known to forage on *Richea scoparia* (Chapter 5; Olsson et al., 2000) their role as pollinator or nectar-robber is still to be determined. In New Zealand, large geckos (*Hoplodactylus* sp.) eat nectar from flowers as diverse as the bottlebrush-like *Metrosideros excelsa*, the large red flowers of *Phormium tenax*, and the small white flowers of *Myoporum laetum* (Whitaker, 1987; Eifler, 1995). Geckos can transport considerable amounts of pollen providing opportunity to effect cross pollination (Whitaker, 1987). As skinks coincide in habitat with numerous epacrid species, it is likely that

associations between skinks and epacrids are more widespread than currently recognised. Of the associations examined here one found that skinks were largely disinterested in foraging on *Acrotriche*, and the other that they foraged extensively on *Richea*, albeit interspersed with foraging for insects (Chapters 3 and 5). Skinks have yet to be confirmed as successful transporters of epacrid pollen between plants.

Nectar quantity and composition in epacrids is largely unexplored and could make an important contribution to our knowledge of pollination ecology. Field observations suggest that *Richea scoparia* and *R. pandanifolia* produce relatively large amounts of nectar. *Richea dracophylla* appears to undertake a secondary nectar presentation combined with rain water in its floral bracts (Fig. 8-1). There is now enough known about animal visitors of epacrid flowers to characterise the nectar profiles of many species, for instance of the bird and insect visited flowers. It may also be possible to refine our information about butterfly and moth visitation as these groups have been suggested to use flowers that have different nectar profiles (Faegri and van de Pijl, 1979), although some epacrids are visited by both. Nectar profiles could also highlight flowers that may be attractive to nocturnal mammals.

Figure 8-1 *Richea dracophylla* with potential secondary nectar presentation in floral bract

To explain the floral diversity of epacrids and how changes in floral morphology may reflect changes in pollinator(s) it is essential to consider the malleability of the floral features and the way that pollinators may perceive the flowers. Overall flower shape is under genetic constraint (Whitney and Glover, 2007), hence the actinomorphic bauplan of epacrids.

Although actinomorphic flowers represent the default floral form, there are numerous examples of independent transition to zygomorphic flowers within other families, and even some changes in the reverse direction (Endress, 2001; Whitney and Glover, 2007). Thus, it is noteworthy that the epacrids have not undergone any changes in their basic architecture. As genetics and neurobiology provide us with a greater understanding of how animals interact with, and influence, the form of plants we will be able to ask more incisive questions informed by the degree and direction of floral trait flexibility.

Overall, there is evidence to support the association of floral traits with particular pollinators. It is probable that these associations have played a major role in the morphological diversification of the epacrids. In addition to red and long corollas relating to bird pollination there are broader examples of flower characteristics relating to pollinators. For instance, the floral trait profiles of *Richea* species were not related to taxonomic divisions but rather to pollination systems (Chapter 5); and the confirmation of sonication in *Sprengelia* also confirmed the independent development of flowers suitable for this pollination type (Chapter 4). Along with collaborators, I have provided the first genus-level molecular phylogenetic hypothesis for the epacrids to use as the back bone for further evolutionary work in this iconic plant family. It has already proved useful in determining some patterns of convergent evolution. However, the story is unfinished. Other flower colours such as purple, green and yellow also show patterns consistent with convergent evolution, but any relationships with particular pollinators are still to be determined. Those parts of the epacrid flora that I have portrayed as unspecialised entomophilous flowers may indeed play host to subtle plant-pollinator relationships that are yet to be uncovered. The role of beetles, butterflies and moths is worthy of closer examination. Keeping in mind the limitations of an inductive approach (Chapter 7), there is much that can be made of forming pollination syndromes specific to groups of closely related taxa using statistical classification techniques. Finally, the hypothesis of evolution of epacrids and their pollination systems that I provide here should be viewed as a foundation work in progress, open for future refinement.

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